A SARCOMA OF THE FOWL TRANSMISSIBLE BY AN AGENT SEPARABLE FROM THE TUMOR CELLS.*

By PEYTON ROUS, M.D.

(From the Laboratories of the Rockefeller Institute for Medical Research, New York.)

PLATES XLVII–LII.

A transmissible sarcoma of the chicken has been under observation in this laboratory for the past fourteen months,[1] and it has assumed of late a special interest because of its extreme malignancy and a tendency to wide-spread metastasis.[2] In a careful study of the growth, tests have been made to determine whether it can be transmitted by a filtrate free of the tumor cells. Attempts to so transmit rat, mouse, and dog tumors have never succeeded; and it was supposed that the sarcoma of the fowl would not differ from them in this regard, since it is a typical neoplasm. On the contrary, small quantities of a cell-free filtrate have sufficed to transmit the growth to susceptible fowls.

EXPERIMENTS.

For the first experiments on this point, ordinary filter paper was used, and the ground tumor was suspended in Ringer's solution. It was supposed that the slight paper barrier, which allows the passage of a few red blood cells and lymphocytes, would suffice to hold back the tumor and render the filtrate innocuous. Such has been the experience of other workers, with rat, mouse, and dog tumors. But in the present instance characteristic growths followed the inoculation of small amounts of the watery filtrate, or of the fluid supernatant after centrifugalization of a tumor emulsion.

These results led to more critical experiments, which will be here detailed. Tumors of especially rapid growth, and young, well-grown fowls of the variety in which the tumor originally occurred, namely, the barred Plymouth Rock, were used throughout.

* Received for publication, February 9, 1911.

[1] Peyton Rous, *Jour. Exper. Med.*, 1910, xii, 696.

[2] Peyton Rous, *Jour. Am. Med. Assn.*, 1910, lv, 1805; 1911, lvi, 198.

Experiment I.—Tumor material from the breast of chicken 92 (tumor generation, 6th A) was ground with sterile sand, suspended in a considerable bulk of Ringer's solution, and shaken for twenty minutes in a machine. The sand and tumor fragments were separated out by centrifugalization in large tubes for five minutes at 2,800 revolutions per minute. Of the supernatant fluid a little was pipetted off and centrifugalized anew for fifteen minutes at over 3,000 revolutions per minute. From the upper layers sufficient fluid for inoculation was now carefully withdrawn. The pure-bred fowls were injected in one breast with 0.2 c.c. of the fluid, in the other with a small bit of tumor tissue. All developed sarcoma at the site of this latter inoculation, and in seven the same growth slowly appeared at the point where the fluid had been injected.

Experiment II.—Tumor from chicken 90 (tumor generation, 6th A) was ground, suspended, and shaken as before. After one centrifugalization the fluid was passed through a Berkefeld filter (No. 2, coarse). Before filtration, it was pinkish yellow, cloudy; afterwards, faintly yellow, limpid. Nine fowls were inoculated with 0.2 c.c. of the filtrate in each breast, and twenty-two more received filtrate in one breast, a bit of tumor in the other. Of the nine, one slowly developed a sarcoma in each breast, and microscopic growths were later found in its lungs. Of the twenty-two receiving both filtrate and tumor, five developed sarcoma where the filtrate had been injected, and these five showed especially large growths from the tumor bit.

The Berkefeld filter employed was afterwards found to be slightly pervious to *Bacillus prodigiosis.* The tumor developing in the fowl injected only with filtrate has been successfully transplanted to another individual.

Experiment III.—The filtrate was similarly prepared except that a small Berkefeld filter (No. 5, medium) was used, impermeable, under the conditions, to *Bacillus prodigiosis.* As before, the filtration was done at room temperature. Fowl 124 (7th generation, A) furnished the material. Twenty chickens were inoculated in each breast with the filtrate, but none have developed tumors.

Experiment IV.—In this experiment the material was never allowed to cool. About fifteen grams of tumor from chicken 140 (7th generation, B) was ground in a warm mortar with warm sand; mixed with 200 c.c. of heated Ringer's solution; shaken for thirty minutes within a thermostat at 39° C.; centrifugalized; and the fluid passed through a filter similar to that used in experiment III. Both before and after the experiment, this filter was tested and found to hold back *Bacillus prodigiosis.* The filtration of the fluid was done at 38.5° C., and its injection followed immediately. In four of ten fowls inoculated into the muscle of each breast with 0.2 to 0.5 c.c. of the filtrate, there developed a sarcoma at one of the points of inoculation; and though the growths required several weeks to appear, their subsequent enlargement was of average rapidity. Pieces removed at operation showed the characteristic structure, and transplantation into other chickens proved successful. Three of the hosts have died, and in two profuse metastases were found. One of the growths furnished the material for experiment V.

Experiment V.—The tumor used resulted from the injection into fowl 180 (8th generation, B) of 0.5 c.c. of the filtrate of experiment IV. Just as in this experiment, the material was ground, suspended, and shaken in the warm, but 300 c.c. of Ringer's solution were used to eleven grams of tumor, and the shaking

was continued only twenty minutes. After centrifuging, the filtration was done at 40° C., through a new Berkefeld filter (No. 5, medium), impermeable, under the same conditions, to *Bacillus prodigiosis.* Ten young hens were inoculated in each breast with 0.5 to 1.0 c.c. of the filtrate; and eleven days later a tumor nodule was just palpable in two of them. One of these growths was at once removed by operation. It took the form of a small raised disc, firm, grayish, and translucent, on the outer surface of the pectoral sheath, presumably at the point penetrated by the injection needle. Its greatest diameter was one and one-half millimeters. In the other fowl the nodule lay deep in the muscle, and seemed little, if at all, larger than that described. In control fowls inoculated with bits of the neoplastic tissue from No. 180, growths measuring from 1.3 to 2.7 cm. in diameter had developed at this time. Twenty-eight days after inoculation, eight of the ten fowls given the filtrate showed tumor nodules, some of them still very small.[3]

Thus the tumor resulting from injection of a filtrate itself furnished material capable of producing tumors after injection.

The importance of the above results depends on the characters of the growth employed. These will now be discussed in detail.

STRUCTURE AND MODE OF GROWTH.

The original tumor was found in the subcutaneous tissue of the breast of an adult, pure-bred hen. The other individuals of the small stock were healthy; and though susceptible normal chickens and chickens with the tumor have been kept together for long periods, no instance of spontaneous transmission of the growth has occurred. The characters of the original mass have been detailed elsewhere.[4] A more general description of the tumor as it has appeared on transplantation will here be given.

Histologically the growth has always consisted of one type of cells, namely, spindle cells, usually in bundles, with a slight vascularizing framework (figure 1). Cell division is usually by amitosis, but mitosis is frequent. Small giant cells, due to the division of the nucleus without corresponding fission of the cytoplasm, are occasionally seen, especially about regions where the growth is degenerating. There is considerable variation in the size of the cells and in the staining qualities of their nuclei, but the growth has not changed in general histological character during its propagation

[3] Later work in this laboratory, by Dr. James B. Murphy, has demonstrated that the tumor can be transmitted by means of the dried and powdered neoplastic tissue, kept at room temperature for many days. The tumors resulting from its injection do not appear for several weeks.

[4] Peyton Rous, *Jour. Exper. Med., loc. cit.*

to the tenth tumor generation. Tumors of the eighth generation, resulting from a cell-free filtrate, resemble the original, except that their cells are plumper, less regular in size, and much more invasive in tendency (figure 2). Figure 3, a drawing from the edge of a growth in the breast that followed injection of a filtrate (experiment IV), illustrates the replacement of striated muscle fibres by tumor cells. Here most of the neoplastic cells are oval, as yet undifferentiated to the spindle form seen in older portions of the growth. Under some conditions, the cells become widely separated by mucigenous ground substance (figure 4). The picture is always that of a spindle-celled sarcoma or myxosarcoma.

Following implantation of a bit of the neoplastic tissue into the breast muscle of a susceptible fowl, a circumscribed nodule shortly becomes evident. It is very firm and definite on palpation, but on section proves unencapsulated, though distinct in appearance from the normal tissue. In general, the neoplastic tissue is gristly and grayish white, with a fine striation on the cut surface. Less often, it is soft, grayish pink, semitranslucent, and friable, or even gelatinous. In the last instance, it may contain much true mucin. As the mass grows large, its scanty, thin-walled blood vessels prove insufficient, and at its center a wide-spread coagulative necrosis, or cystic change takes place, the latter not infrequently as the result of hemorrhage. The cysts are filled with serous or ropy fluid, often colored with blood pigment; and polypoid extensions into them of the tumor are not rare. Continuing to grow, the mass extends to the muscle-sheath and perhaps through this to connective tissue and skin. Infiltrating the latter, it may spread rapidly *en cuirasse;* but ulceration is seldom seen. Soon the whole of the inoculated breast is occupied by a bulging, rounded, firm growth (figure 5); and the host rapidly emaciates, becomes cold, somnolent, and dies. In many cases the viscera, especially the lungs, heart, and liver, are the site of discrete metastases, gristly and firm, like the primary growth. Those on the surface of the liver may be umbilicated (figure 6).

TUMORS RESULTING FROM A FILTRATE.

The tumors which result from the injection of a cell-free filtrate take much longer to appear. The inoculation into the breast muscle

of a bit of tumor tissue one millimeter in diameter may give rise in the course of a week to a growing nodule 1.5 centimeters broad; whereas, following the injection of a filtrate, not the slightest trace of tumor is palpable for from ten days to three weeks. Then one or two minute, shotty bodies can be felt, and soon the characteristic mass develops. At first it is ovoid or spherical in shape, just as though it had arisen from an introduced bit of neoplastic tissue. In one case a flat, irregularly branching mass, flame-shaped, so to speak, developed in the sheath of the pectoral muscle at the point injured by the injecting needle. Many of the injected fowls in which no growth appeared in the breast muscle have been carefully examined at autopsy for tumors elsewhere, but none have been found. Some with a growth in the breast have developed after a time others in the viscera, probably the usual metastases, to judge from size and distribution. Careful note has been kept as to whether the tumors resulting from a filtrate injection grow more slowly than usual. This has been found to be the case. Following their tardy appearance, a considerable proportion of them grow slowly as compared with control tumors resulting from implantation.

INFLUENCE OF THE HOST.

The stock in which the original tumor occurred consisted of fowls of one pure-bred variety, the barred Plymouth Rock. The first transplantation was made to chickens from the same setting of eggs as the individual with the tumor, and the next successful one to less closely related members of the same stock. Bits of the growth were placed in the breast muscle by means of a trocar, a procedure adopted as the routine. Repeated unsuccessful attempts were made to transfer the growth to chickens resembling the tumor stock, and of similar variety, but obtained from another source, and probably not pure-bred (chart 1). Attempts at this time to transfer it to chickens of another variety and to pigeons and guinea pigs also failed. Recently the sarcoma has increased in malignancy and has gained the power to grow in chickens of other kinds. Yet in them it develops slowly, or long remains stationary; and it has never been successfully transmitted to other species, although pigeons, ducks, rats, mice, guinea pigs,

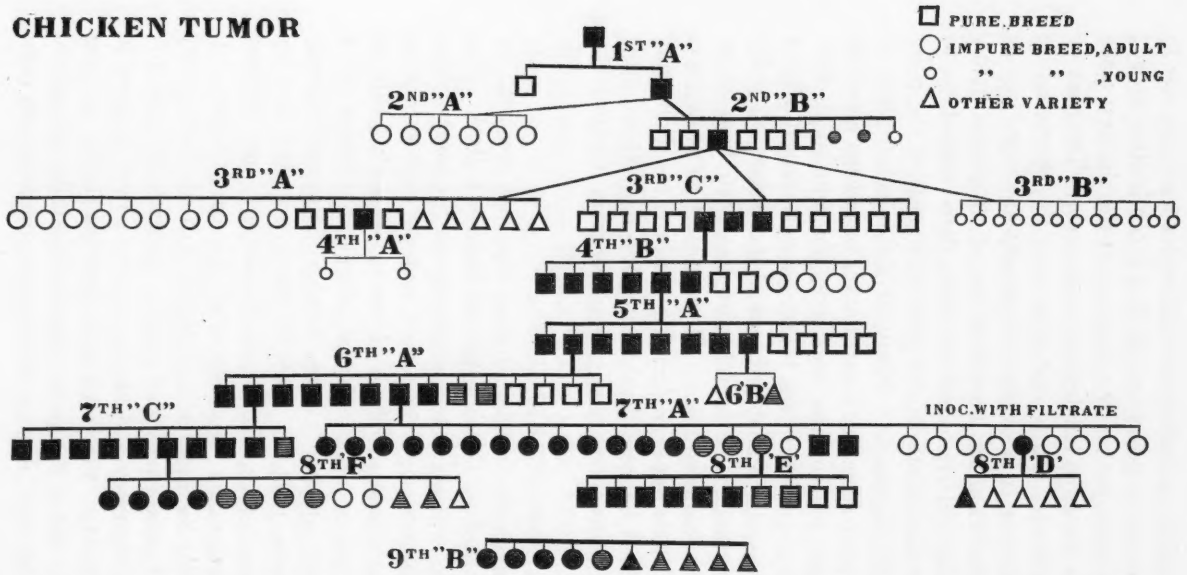


CHART 1. This chart of the first eight generations of the sarcoma illustrates the influence of the variety of the host. The blackened symbols represent those hosts in which the tumor grew, the cross-barred ones those in which it appeared, but remained stationary, or retrogressed. The fowls used were barred Plymouth Rocks of pure breed, barred Plymouth Rocks bought at random and hence presumably not pure-bred, and other chickens of heterogeneous sort and appearance. All were inoculated with bits of the tumor, except nine individuals of 7th generation A, which received portions of a Berkefeld filtrate. A number of the series of the 7th and 8th generations are omitted from the chart, because in them the conditions were irregularly modified. The chickens of 9th generation B were inoculated with material from a member of one of these series. Here the importance of the host's variety is still evident, though the cross-barring of the symbols now means that the tumor grew slowly, not that it remained stationary or retrogressed.

and rabbits have recently been inoculated. At present it grows in 80 to 100 per cent. of barred Plymouth Rock fowls and is especially active in young ones. Retrogression of a developed nodule, fairly frequent at first, is now rare, save when the host is sick. Intercurrent illness of the host may check the further development of a nodule two or three centimeters in diameter, and may even cause its retrogression (chart 2). Should the host's health return, the tumor may reappear and grow rapidly. Retrogression in the healthy host confers resistance, so that further inocluation with active material does not result in growths. A fowl which has once failed to develop a tumor usually proves resistant on later inoculation. Resistance, therefore, is both induced and, apparently, natural also.

EFFECTS OF TRANSPLANTATION.

The original tumor had been under observation for two months before it was brought to the laboratory, and had grown slowly during that period. With repeated transmission the rate of growth, as well as the percentage of successful transplantations, has increased; and the period which elapses between implantation and the appearance of the new sarcoma as a palpable mass has been reduced from about four weeks to four or five days. The tumor obtained from the first inoculation required seventy-one days to reach a size of 5.0 by 3.3 centimeters, and to affect seriously the health of the host. But in later generations, produced by a similar method of inoculation, the appearance and development of the tumor have become progressively more rapid. Growths ten or twelve centimeters in length by six in width are now found three weeks after inoculation of a bit of neoplastic tissue, two millimeters in diameter; and often death of the host ensues within twenty-six to thirty days, all told.

Repeated transplantation has also greatly increased the frequency, extent, and rapidity of metastasis formation. The death of the original fowl was hastened by a successful intraperitoneal implantation with its own growth, and the autopsy did not disclose any nodule suggesting a metastasis. The tumor fowl of the first generation was killed after seventy-two days, and had, in its heart, one small secondary growth. Metastasis did not take place in the second

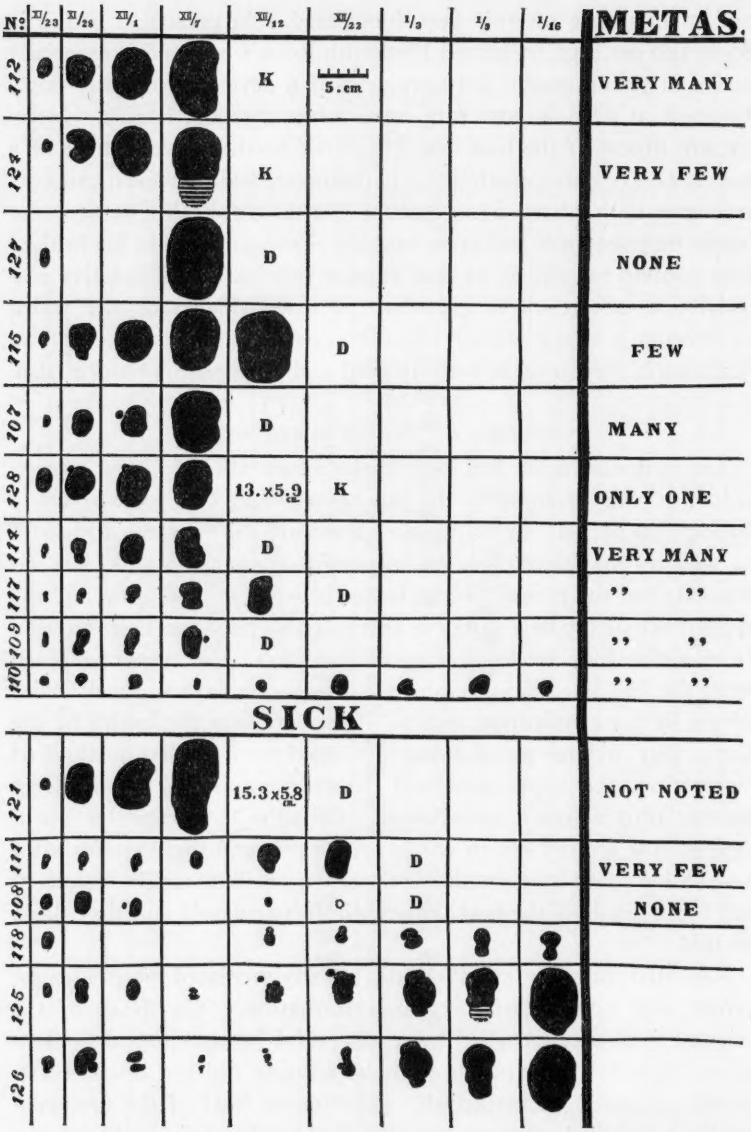


CHART 2. The effect upon the tumor's growth of intercurrent illness of the host. The figures in the first vertical column are the serial numbers of the

fowls, those in the first transverse column refer to the dates on which the tumors were measured. In the last vertical column, record is made of the visceral metastases at autopsy. "D" or "K" indicates that the fowl died or was killed.

The fowls (7th generation A) were all inoculated with the same material, and the first measurements were taken eight days later. Within two weeks, six of the hosts had fallen ill of an intercurrent disease, characterized by rhinitis, conjunctivitis, and marked depression and emaciation. Tumors had already developed in all six, but now retrogressed in four, completely disappearing in two. The fowls were placed under better conditions and three recovered health. In two, the tumor reappeared and grew rapidly.

tumor generation; but in the third it was frequent; and in the later ones it has been present in a majority of hosts dying of the growth. In the seventh generation the average time required after inoculation for the dissemination and development of secondary nodules was approximately half that demanded in the third. Metastasis takes place first and most often to the lungs (figure 7). In the earlier generations the heart was especially effected (figure 8), though nodules were found in the liver, much less frequently in the kidneys (figure 9), and occasionally in the intestine, mesentery, pancreas, gizzard (figure 10), gall-bladder, and lymph-glands. Of late the heart has held fewer nodules, and they have been frequent in the liver. The spleen was exempt from them until the seventh generation, when in two cases out of twenty-five it contained nodules. In these two cases the tumor showed itself in other ways especially malignant. The spleen has since been often affected.

MODE OF METASTASIS FORMATION.

The question as to how metastasis of the chicken sarcoma takes place has great importance. For it might be supposed that the agent which suffices, independent of the cells, to transmit the tumor to new individuals would itself cause secondary masses in the host. Perhaps sometimes it does act alone to produce such masses, though we have met no instance in which this can be affirmed. On the contrary, the findings all indicate, as with the sarcomata of man, that the metastases result from a distribution of tumor cells, usually by way of the blood. As has been said, the relations of the sarcoma to the blood stream are very intimate; quite large vessels walled with only a layer of endothelium exist within the growth, and in their

neighborhood cell proliferation is at its height. Instances in which the neoplastic tissue has penetrated a vessel wall are frequent (figure 9), and sometimes a strand of the growth, quite bare of endothelium, extends for a considerable distance in the blood stream (figure 8). The secondary growths in the viscera are first evident as small, approximately spherical groups of cells with a blood-vessel in the midst, occluded by tumor tissue (figure 7). The cells are of one type, in active mitotic and amitotic division, unsurrounded by inflammatory reaction. This is true of metastases consisting of only three or four cells. To recognize a single neoplastic cell lodged in a capillary has thus far proved impossible because the morphology of the single cell does not sufficiently identify it; yet what would appear to be such emboli are frequent in the pulmonary tissue. The question of the transplantability of the tumor cells has been settled in the affirmative by an examination of grafts of the neoplastic tissue removed shortly after implantation.

Metastasis by way of the lymph-stream occasionally occurs: the glands along the great vessels above the heart have been found enlarged and entirely replaced by sarcomatous tissue. Contact metastasis is very frequent. For example, a mass in the breast may penetrate the sternal membrane and give rise to nodules on the liver surface opposite. In the viscera the tumor preserves its histological character, but its arrangement is influenced to a certain degree by preëxisting structures. In the lung the pattern of the alveoli may be perpetuated in tumor cells, as is true also of the striped muscle (figure 3). The skin may be infiltrated and tightly stretched. Frequently the growth recurs in wounds made to remove subcutaneous grafts, and here its presence need not greatly impede healing. The early stages in the development of a sarcoma caused by a cell-free filtrate are difficult to obtain. When such a growth becomes palpable, it is already one to two millimeters in diameter, and histologically no more enlightening than a metastasis or a graft of the neoplastic tissue would be.

THE TUMOR CELLS ARE TRANSPLANTABLE.

A study of many grafts removed at short intervals from the connective tissue has shown conclusively that the inoculation of a

bit of the sarcoma into a susceptible fowl results in an actual transplantation of the neoplastic cells and growth from them. For the first two or three days after implantation, the graft is unattached to the host tissues, but then it unites with them, is vascularized, and begins to enlarge and to invade the surrounding parts (figure 11). Usually a few small mononuclear cells (lymphocytes) collect at its edge, but no other cellular reaction follows that is due to the initial trauma. Indeed, about metastases a cellular reaction is often completely absent. Unless the graft is very small its central part dies before vascularization can take place. There remains, however, a living periphery distinct from the normal tissues of the host, and soon this is vascularized and strands of the spindle-shaped cells can be seen growing out from it. The findings do not in the least suggest that the tumor is transmissible apart from its cells. Certainly it is transplanted easily and is, at present, best propagated by this means.

The death of grafts of the tumor in fowls with a natural or acquired resistance takes place in one of two ways. The implanted tissue may fail entirely to be vascularized, and its death results after some days, during which a zone of living cells persists at its periphery (figure 12). Or it may be vascularized and grow for a brief period, dying at last in the midst of an accumulation of lymphocytes. The first process is seen especially in regions poor in connective tissue and blood-vessels, and may occur there even in susceptible hosts. The second process is the one found at the edge of retrogressing tumors. A more detailed account of the fate of early grafts has been reserved for another paper. The findings here briefly described are those made familiar to tumor workers by a study of rat, mouse, and dog tumors.

Cultures from the growth upon many media have repeatedly been taken; but with the exception of a large post-mortem bacillus once obtained, they have remained sterile as regards bacteria. Portions of the filtrate and fresh smears from the tumor surface have been examined with the dark-field microscope, but neither this nor the various histological procedures applied to the neoplastic tissue has disclosed anything which can be recognized as a parasitic organism.

DISCUSSION.

It is evident from the foregoing description that our tumor of the fowl possesses to a marked degree those characters of morphology and behavior which distinguish the true malignant neoplasms, especially the sarcomata. It is formed of a single type of cells, only slightly differentiated, resembling young connective tissue cells, and possessed of an enormous proliferative energy which is exercised to the detriment of the surrounding tissues and eventually of the entire host. Growth takes place through infiltration and replacement of normal structures, as well as through expansive enlargement. Metastasis by way of the blood stream is common, rarer by the lymphatics; and, to judge from histological evidence, the transportation, lodgment, and growth of tumor cells is wholly responsible for the secondary nodules. Indeed, a general histological study of the sarcoma would not lead one to suspect that it can be transmitted by another means than a transplantation of cells. When a small bit of the neoplastic tissue is placed in a new and susceptible host, most of its cells survive, are vascularized, and by their proliferation give rise apparently to all of the growth. In a resistant host, the graft soon dies and no tumor follows. One would suppose that the sarcoma developed only "aus sich heraus," to use Ribbert's phrase. But histological pictures are not decisive upon this point. Since the growth is transmissible by a cell-free filtrate, it seems not unlikely that in its neighborhood the connective tissue cells of the host undergo a neoplastic change.

A feature of the transmissible tumors, which has largely drawn the attention of cancer workers and has modified current theories of cancer origin, is their striking dependence for a successful transplantation on the character and condition of the individual host. It is a dependence similar to that shown by transplanted normal tissue, and apparently the same laws largely influence both. This trait of tumors is illustrated exceptionally well by the chicken sarcoma. During a considerable period, it could be propagated only in fowls of precisely the sort in which the original growth occurred (chart 1); and even now it succeeds best in these. It has never been successfully transmitted to birds of other species, or to mammals. Young fowls are the most favorable hosts; and healthy,

well-nourished ones proves more susceptible than the thin and ill. Indeed, intercurrent illness of the host may cause the sarcoma transiently to disappear (chart 2).

The above traits have figured largely in current discussions on cancer etiology, and most of them have been regarded as evidence against a specific cause for the disease, extrinsic of the cells. Such evidence is void, now that a growth has been found possessing the traits mentioned, yet transmissible independently of its cells. This fact, and not the problem of how to classify the growth, merits attention. Nevertheless, a passing reference should perhaps be made to the ill-defined group of pathological products called granulomata, with which this neoplasm of the fowl may by some be classed, owing to its transmission by an agent separable from the tissue cells. None of the granulomata has the tumor characters, and none is known to be transplantable. The present growth fails to resemble any granuloma thus far described; whereas it fulfills all the conditions for identification as a tumor.

The first tendency will be to regard the self-perpetuating agent active in this sarcoma of the fowl as a minute parasitic organism. Analogy with several infectious diseases of man and the lower animals, caused by ultramicroscopic organisms, gives support to this view of the findings, and at present work is being directed to its experimental verification. But an agency of another sort is not out of the question. It is conceivable that a chemical stimulant, elaborated by the neoplastic cells, might cause the tumor in another host and bring about in consequence a further production of the same stimulant. For the moment we have not adopted either hypothesis.

The ultimate significance of these unusual findings can hardly be well discussed until more data are obtained through experiment, especially through carefully devised experiment with the tumors of other species of animals. For it is quite possible that the failure to separate from these growths an agent causing them may be traceable to some interference with the conditions under which this supposititious agent can exist alone, or reproduce the growth in new hosts. Work along the line indicated is under way in this laboratory.

EXPLANATION OF PLATES.

Unless otherwise indicated, the sections are stained with Delafield's hematoxylin and eosin. In each case the tumor generation and series are given, followed by the number of fowl from which the specimen came; thus, 7th A, No. 117.

PLATE XLVII.

FIG. 1. An area in the original growth. Necrosis is present at one side.

FIG. 2. 8th B, No. 177. Part of a tumor in the left breast, resulting from the injection of the filtrate of experiment IV. The muscle fibres are largely invaded and replaced by tumor cells. Methylene-blue and eosin.

PLATE XLVIII.

FIG. 3. 8th B, No. 177. A drawing which further illustrates the invasion and replacement of muscle fibres by tumor cells. The preparation is from the same growth as Fig. 2.

FIG. 4. 8th B, No. 173. Myxomatous growth caused by the filtrate of experiment IV.

PLATE XLIX.

FIG. 5. 5th A, No. 82. Growth in the left breast of a chicken, resulting from the implantation of a small bit of tumor tissue fifty-two days previously. The skin and connective tissue covering have been removed. The length of the mass is fourteen centimeters. In the right breast is a nodule that resulted from injection of the fluid supernatant after centrifugalization of a tumor emulsion.

FIG. 6. 7th A, No. 117. Metastases from a growth in the left breast. The chest, and abdominal wall, and about half of the breast tumor have been cut away to expose the viscera. The lungs, much enlarged, are crowded with discrete tumor nodules. The metastases on the surface of the liver are umbilicated and surrounded by a zone of dilated blood-vessels. The duration of the disease was thirty-seven days.

PLATE L.

FIG. 7. 6th A, No. 102. A small metastasis in the lung, with an occluded blood-vessel at its center. The tumor cells have only short, blunt processes, a variation that is not infrequent. The picture is complicated by the presence of many nucleated red cells.

FIG. 8. 4th B, No. 63. Growth of a tumor of the heart wall into the ventricular blood. A tongue of myxomatous tumor here extends between two trabeculae of heart muscle. The dark mass at its end consists of nucleated erythrocytes. The transverse rent in the heart muscle above the tumor is an artefact.

PLATE LI

FIG. 9. 4th B, No. 63. Kidney. Extension of the tumor through the wall of a vein.

FIG. 10. 7th A, No. 116. Margin of a metastasis in the muscle of the gizzard. At one corner some uninvaded tissue is seen. Note the complete absence of any cellular reaction about the tumor.

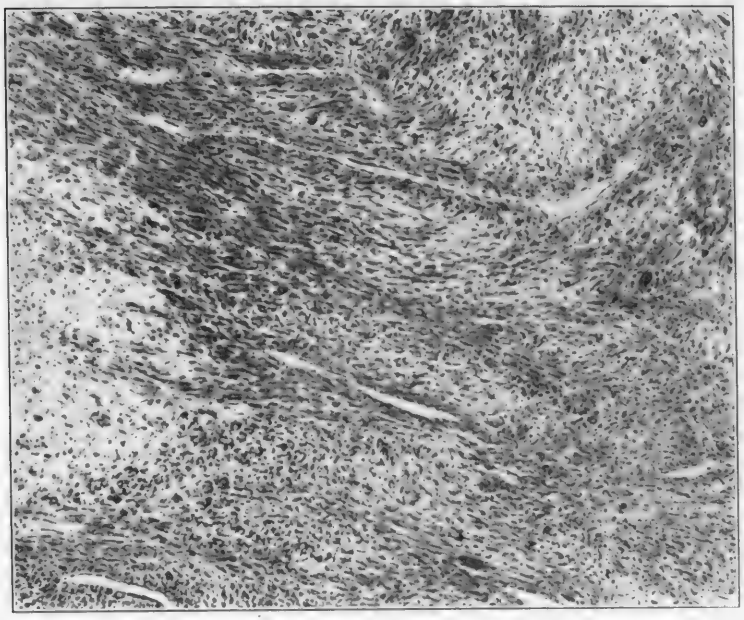


FIG. 1.

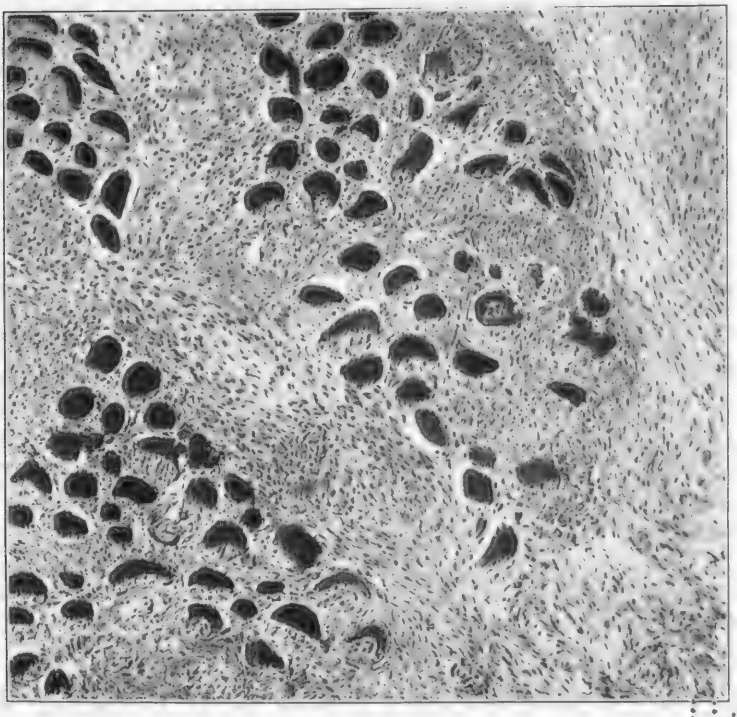


FIG. 2.

U of M

Mau

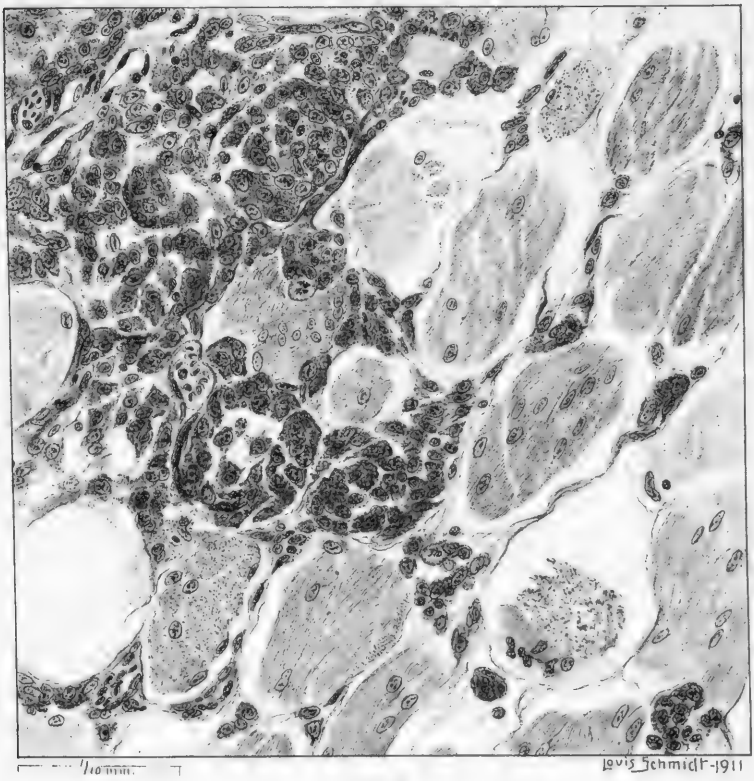


FIG. 3.

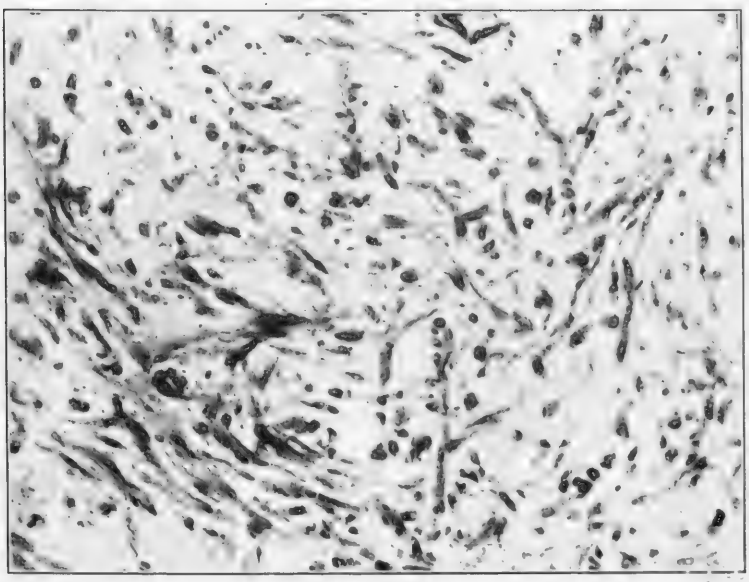


FIG. 4.

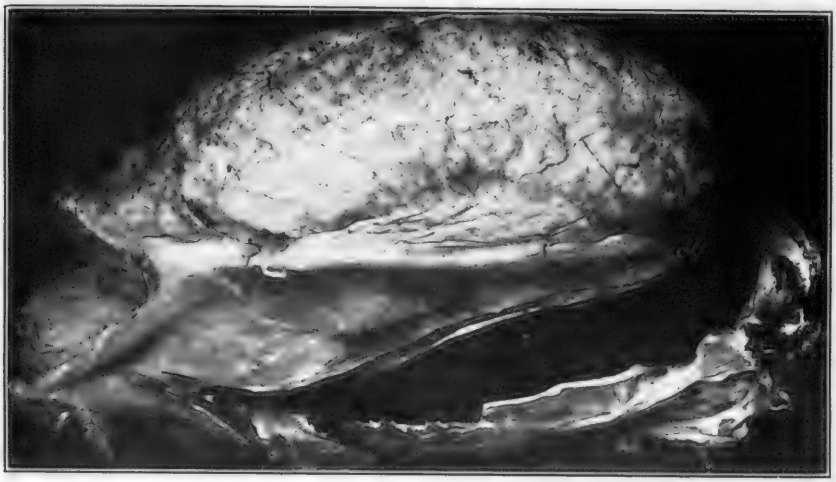


FIG. 5.

FIG. 6.

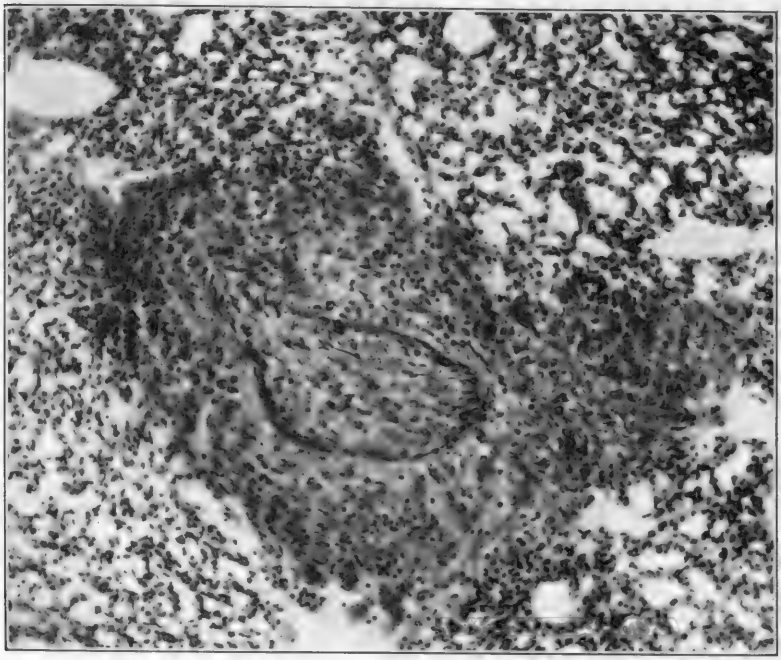


FIG. 7.

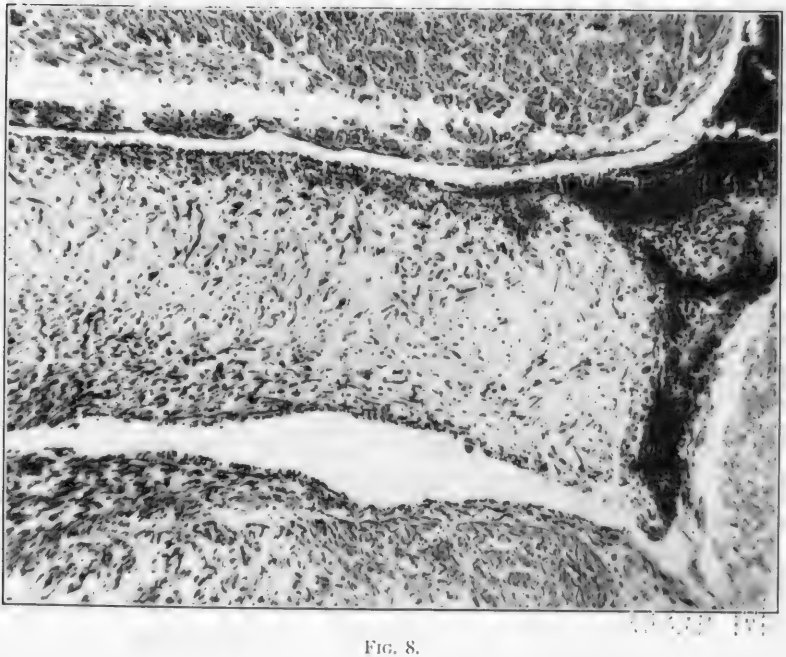


FIG. 8.

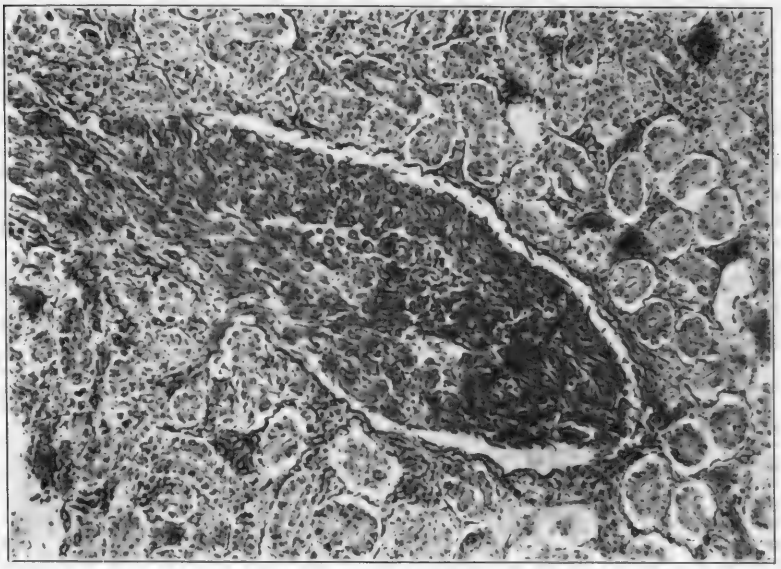


FIG. 9.

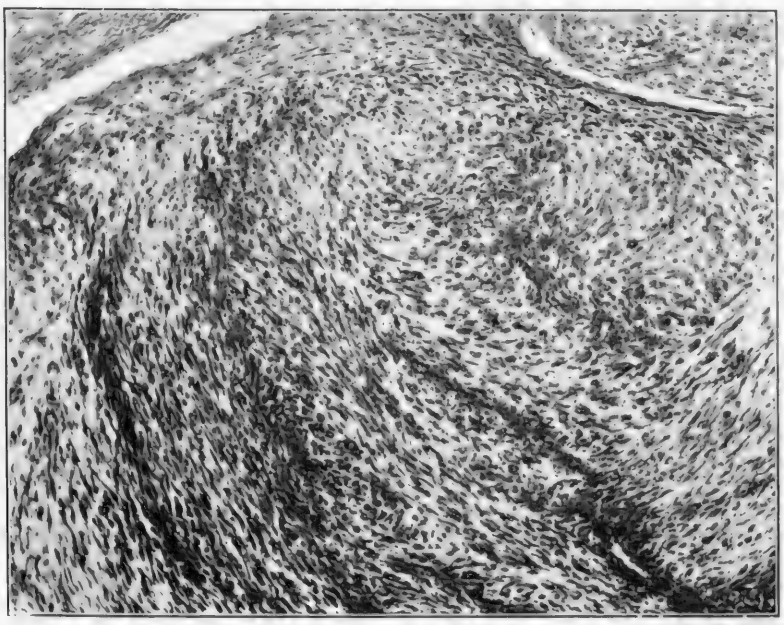


FIG. 10.

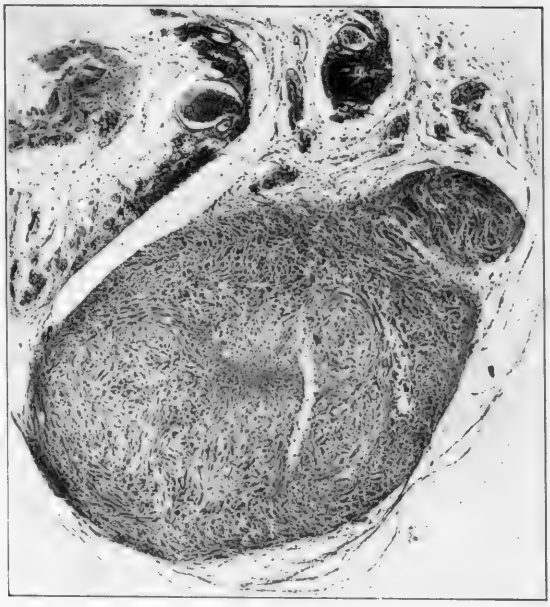


FIG. 11.

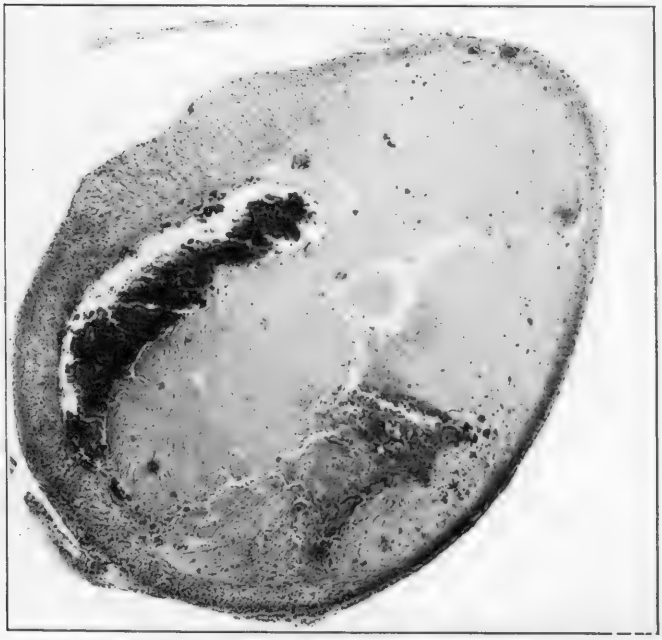


FIG. 12.

PLATE LII.

FIG. 11. A graft of the sarcoma removed with the surrounding tissue four days after implantation in a susceptible host. Already it has united at two points with the host tissue and vascularization is in progress, though too late to prevent necrosis at the center of the graft.

FIG. 12. A similar graft removed from a resistant host nine days after implantation. Despite the long period, the graft is joined to the host only by two thin strands of connective tissue shown at either end. It is unvascularized and necrotic save for a thin peripheral zone of the characteristic cells, which is considerably infiltrated by lymphocytes.

THE TREATMENT OF YAWS (FRAMBŒSIA) WITH ARSENOBENZOL (SALVARSAN).*

By RICHARD P. STRONG, M.D.

MANILA, P. I.

PLATES LIII AND LIV.

Yaws is a highly contagious and disfiguring disease very prevalent in the tropics and believed now to be caused by a specific spirochæte—the *Treponema pertenuis* of Castellani. Although some writers have held that yaws and syphilis are merely two manifestations of the same disease, it can now be stated that the weight of opinion is against that view and in favor of the view that they are distinct diseases. With this latter conclusion the experimental work of the last few years is in harmony.[1] Monkeys can be inoculated with yaws as with syphilis; and monkeys which have recovered from syphilitic lesions are still susceptible to inoculation with yaws, although they are not reinoculable with syphilis. Moreover, *Treponema pertenuis* is distinguishable morphologically from *Treponema pallidum;* and thus while it can be affirmed that both syphilis and yaws are caused by spirochætal organisms, it can also be stated that the spirochætæ belong to different species. All the evidence is, therefore, to the effect that the diseases are distinct entities.

Hitherto, the treatment of yaws has been highly unsatisfactory. In the first place, the usual treatment employed must be continued over long periods of time to attain any permanent result. While this is possible in some cases among the better class of patients, it is nearly impracticable with the class of dispensary and hospital patients, and among the lower classes of natives it is wholly impracticable. There would appear to be no specific treatment for

* The substance of this paper was read at the meeting of the Manila Medical Society, September 5, 1910, at which one of the cured patients was exhibited. Received for publication, December 29, 1910.

[1] This is the inevitable conclusion from the experiments of Charlouis, Neisser, Baermann, Halbertstädter, Ashburn and Craig, Levaditi, and others.

yaws. Such eminent authorities as Manson, Scheube, Castellani, Plehn, Firth, and Jeanselme are unanimous in designating the present methods as very unsatisfactory. Probably, indeed, none of the older drugs—mercury, arsenic—actually influences the duration of the disease. Potassium iodid, which seems to accomplish more, is notably uncertain in its action. The eruption will sometimes disappear rapidly when iodids are administered; but even in such cases, during the employment of the drug, fresh eruptions appear.

ARSENOBENZOL A SPECIFIC REMEDY FOR YAWS.

A few months ago Professor Ehrlich kindly sent me a supply of dioxydiamidoarsenobenzol, or "606," or, as it is now designated, arsenobenzol or, preferably, salvarsan, the last being the name under which the drug has been introduced into commerce, to be employed in the treatment of syphilis. So many publications based on the employment of the drug in syphilis have already appeared that I shall not deal with that subject at the present time. In view, however, of the favorable results obtained with the drug in the treatment of syphilis, I determined to test its effects in yaws. This decision seemed warranted by the close morphological and biological relationship existing between the spirochætæ causing the two diseases and by the fact, established by Ehrlich and Hata,[2] that fowl spirochætosis is favorably affected by the drug. Moreover, Iversen[3] had already applied the drug successfully to the treatment of relapsing fever in man, in which condition the spirochætæ are rapidly destroyed and the disease is cured.

Up to this time twenty-five cases of yaws have been treated with arsenobenzol. The full reports on the cases is being reserved for the present in order to ascertain whether any relapse will occur. So far there have been no evidences of a relapse and the cases have remained cured, although many of them were treated four months ago or longer.

The form of preparation employed was that of the alkaline solu-

[2] Ehrlich and Hata, Die experimentelle Chemotherapie der Spirillosen, Berlin, 1910.

[3] Iversen, *München. med. Wchnschr.*, 1909, lvi, 1785.

tion, which was injected deeply into the gluteal muscles. On the day following the injection, some reddening, swelling, and induration of the skin and deeper tissues were usually present; but in no instance did suppuration develop, and pain, when complained of, was stated not to be severe. Among those treated were several children who did not seem to suffer pain either during or after the injection, although they were noted to be less lively than usual for about twenty-four hours after the treatment.

A single injection, has, up to the present, proven sufficient to bring about a cure of the lesions of yaws, and this notwithstanding the fact that in a number of instances tertiary lesions with ulceration existed. No other form of treatment, either local or general, was employed along with the arsenobenzol. The effects of the drug are exercised quickly. Three or four days after its administration the granulomatous lesions begin to change and grow smaller, and in from ten to twenty days they have usually disappeared and left behind a smooth pigmented surface of skin. The effects have, indeed, been both striking and surprising, and in some cases, in contrast with the other modes of treatment, almost magical. In from twenty-four to forty-eight hours after the injection, the margins of the granulomatous lesions are reddened and the centers rendered of a bluish hue, both the results of congestion. The spirochætæ soon disappear and absorption of the lesions progresses quickly. The skin finally resumes its normal color.

Although in none of the series of cases reported was a second dose of the drug given, I believe that in severe cases of long standing in which the ulcerations are extensive, a second injection could be administered with advantage about three weeks after the first. I have employed from 0.25 to 0.3 gram of the drug in children, and from 0.4 to 0.5 gram in adults. I should add that I have never observed any unfavorable constitutional symptoms follow the administration of the drug; and, on the other hand, that I have seen the general health improve rapidly after the disappearance of the eruption.

The accompanying photographs (figures 1, 2, 3, and 4) illustrate the conditions before and after the administration of the drug. They represent a very few of the results obtained.

FIG. 2.

FIG. 1.

Fig. 4.

Fig. 3.

Use M

Finally, I should add that while the studies reported were in progress, the papers of Nichols[5] from the Rockefeller Institute for Medical Research came to my notice. In these papers are described for the first time the experimental production of testicular lesions in the rabbit, containing numerous spirochætæ, by means of material from the lesions of yaws obtained from a colored soldier who had returned to the United States, and the rapid cure of those lesions by means of a single injection of arsenobenzol.

EXPLANATION OF PLATES.

PLATE LIII.

FIG. 1. Case I. Before treatment. Granulomatous lesions on face, chest, arms, legs, etc.

FIG. 2. Case I. Twelve days after injection of arsenobenzol. All the lesions have disappeared and their site is indicated by smooth, pigmented skin.

PLATE LIV.

FIG. 3. Case II. Before treatment. Granulomatous lesions on face, trunk, arms, legs, and feet.

FIG. 4. Case II. Nine days after injection of arsenobenzol. All the lesions have disappeared, the skin has become smooth, and only some pigmented areas remain.

[5] Nichols, *Jour. Am. Med. Assn.*, 1910, lv, 216; *Jour. Exper. Med.*, 1910, xii, 616.

CULTIVATION IN VITRO OF THE THYROID GLAND.*

By ALEXIS CARREL and M. T. BURROWS.

(*From the Laboratories of the Rockefeller Institute for Medical Research, New York.*)

Plates LV–LVII.

In a previous paper[1] we have described in full the method employed by us in cultivating tissues and organs of cold- and warm-blooded animals *in vitro* and the general results thus far secured with the method. In this paper we wish to present in greater detail the experiments performed with the thyroid gland.

We cultivated the thyroid gland for the first time on September 22, 1910. Since then we have cultivated this gland, taken from mammals, many times. Small fragments of the gland were extirpated from living and anesthetized dogs, cats, and guinea pigs, and cultivated in the plasma obtained from the same animal or an animal of the same species. These cultures are distinguished as autogenic and homogenic. In all, we have now made fifteen series of cultivation experiments with the gland, each of the series being composed of from four to thirty separate cultures.

PRIMARY, SECONDARY, AND TERTIARY CULTURES, AND SECOND GENERATION OF THYROID CELLS.

A primary culture consists of the growth in plasma of a fragment of the gland obtained directly from an animal. A secondary culture is secured by extirpating a fragment of the gland growing in the primary culture and transplanting it to a new plasmatic medium. When a living fragment of thyroid is removed from a primary culture and transferred to a fresh medium, growth often begins anew. A fragment which has produced chiefly or only connective tissue in a primary culture may, on being transferred, produce a continuous layer or tubular formations of epithelial cells

*Received for publication, February 7, 1911.

[1] *Jour. Exper. Med.*, 1911, xiii, 387.

in the secondary culture (figure 1). In the course of this process of growth, the original fragment becomes more and more translucent, apparently from the out-wandering of the constituent cells. A culture, therefore, contains emigrated as well as proliferated cells. The secondary cultures are best obtainable from primary cultures that are still growing actively. From the third to the eighth day the period is favorable; later it is unfavorable. The second generation of cells may be abundant or sparse or fail altogether to appear. In some instances the plasmatic medium may be rapidly and generally invaded with cells that present no distinct outlines and are detachable by reason of the bright and refractive granules that they contain. A tertiary culture is made in the same way from a secondary culture. In a few instances a fresh plasmatic medium was inoculated with cells from a thyroid fragment grown outside the body. The attempt rarely succeeds because it is very difficult to prepare a suitable section of the old clot, and the cells contained in it are therefore injured or killed by the act of transfer.

Another and more successful procedure for obtaining a second generation of growing cells is to extirpate the original fragment of tissue from the middle of the clot and to fill the resulting space with fresh plasma. The cells contained in the old plasma multiply and invade the new. The cultivation of cells in series was attempted in a few instances only, and in most of these a second generation could be secured. Thus far we have not secured a third generation of the thyroid cells. We do not, however, believe this to be impossible, as the number of experiments performed by us is too few to be conclusive. The technique of cultivation of tissue cells in series is far from being worked out. Indeed, the results obtained in primary cultivations are subject to wide fluctuations depending on the exactness of the technique. The positive results increase in direct ratio to the precision with which the experiments are conducted. In January, 1911, we secured eighty per cent. of positive results, while in September and October, 1910, when the technique was less exact, the percentage was less than fifty.

Failure of the thyroid tissue to grow may be due to one or more causes. When the medium has coagulated properly about the fragment and growth does not take place, it is probable that undue

crushing or drying of the tissue before transplantation has occurred, or that a trace of antiseptic may have entered the tissue or plasma. When sudden cessation of the active growth takes place during the first few days, the plasma has generally suffered drying or bacterial infection, or the temperature of the oven has changed markedly. Bacterial infection is usually attended with liquefaction of the medium, but liquefaction may arise from other causes.

THE THREE PERIODS OF A CULTURE.

Almost all our observations have been upon primary cultures of the thyroid gland, the phases of which can be divided artificially into three periods; namely, latency, growth, and death.

1. The latent period covers the time from the inoculation of the fragment in the plasmatic medium until the appearance of the first cells. Note must first be taken of the appearance of the culture immediately after its preparation: the thyroid fragment appears as an opaque body with more or less sharply defined edges, lying within a clear medium. Within the fragment it is possible to distinguish, under the microscope, between the glandular and the supporting tissues. The period of latency endures from twelve to seventy-two hours, according to the age of the animal supplying the fragment, and some other conditions. In the case of a six day old kitten, growth began in twelve hours; in that of a young dog, it began after twenty-four to forty-eight hours. In the case of adult animals, two to three years old, the period extended to forty-eight or seventy-two hours.

2. The period of growth varies considerably. It may extend to eighteen days and is determined, doubtless, by many factors, of which few or none have been worked out up to the present. We shall confine ourselves to a description of the new cells produced in the culture.

The earliest cells produced are fusiform or polygonal and wander freely from the fragment of tissue into the medium. Some of the later cells tend to form continuous layers that spread from the fragment into the plasma (figure 2). We do not possess at present any criteria for determining absolutely the nature of the proliferated cells, but we believe that they consist of connective tissue

and epithelial cells. The earlier cultures of the thyroid of the dog and chicken yielded exclusively a connective tissue growth. The cells were isolated and elongated or irregular, possessed a large clear nucleus with one or two nucleoli and protoplasm containing many granules which were grouped about the nucleus or filled the cytoplasm. These cells invaded the medium either as isolated single cells or in rows or chains of cells (figure 3). Ultimately their processes united to form an open network. They did not give rise to continuous layers, as we have observed the cells of the epidermis to do. On the other hand, their multiplication goes on rapidly so that they come in a few days to occupy several planes of the plasma. In a few instances they surrounded the fragment concentrically and produced a kind of dense network or capsule.

The cells of another type were polygonal in form and appeared somewhat later than the fusiform cells. They presented less distinct outlines and a finely granular protoplasm surrounding a large clear round nucleus which in turn contained one or two opaque nucleoli (figure 4). These cells differ from the others in remaining in a community and not wandering separately into the medium, and in producing sometimes tubular formations (figure 5) and sometimes continuous layers (figure 2). Moreover, these cells grow from the edges of the fragment as far as the upper surface, and in a single plane. In one instance, the tubular proliferation was traced to the circumference of a thyroid vesicle which formed its base. In some instances the growth was cup-shaped, and later budding occurred so that ramifying tubules were produced. Hence we think that there is good reason to regard these cells as of epithelial origin.

The foregoing descriptions refer to the fresh, living cells. The cultures can be fixed at will and stained in the usual manner. Hematoxylin and eosin stained preparations showed that the layers and tubules of cells consisted of a faintly stained cytoplasm containing obvious nuclei but showing only slightly the demarcation between the cells (figure 6). Now and again the limits defining the cells could be made out and were similar to those of the epidermis.

The period of active growth of a thyroid culture is from six to eight days. When it exceeds this, the proliferation of cells goes

on very slowly. In a culture that remained alive for eighteen days, there was slight multiplication of cells after the tenth day. During the proliferation of cells, the original fragment becomes progressively clearer and the alveoli are easily visible, an effect produced probably by the wandering of many cells into the medium. As respects rate of growth, the thyroid equals that of the ovary, testicle, and kidney, and exceeds that of the peritoneum and cartilage. The medium becomes progressively darker as growth proceeds, and the fibrin network more apparent. The plasmatic clot tends to rarefy at the edges of the tissue, and at times it retracts so that a clear area of fluid surrounds the fragment. This occurrence prevents further growth.

3. The death of the culture takes place after ten to eighteen days, that is, after a complete cessation of the multiplication of cells. Coincidently, the cytoplasmic granules coalesce and increase in number. The outlines of the cells grow faint and finally disintegration of the cells takes place. The death of the culture is bound up with changes induced in the plasma by the growing cells. This is shown by the fact that on secondary transplantation the cells continue to multiply. It may be caused by exhaustion of the nutriment or by accumulation of metabolic products or by both of these factors together.

CONCLUSION.

The thyroid gland of mammals can be cultivated outside the body. The proliferated elements consist of connective tissue and epithelial cells, the former predominating. The cells survive in cultures for two weeks or longer, which period can be increased by secondary and sometimes by tertiary cultivations. It is to be noted that the method of growing *in vitro* organs such as the thyroid gland may come to be used with advantage in the study of the substances concerned with the internal secretion of certain glands.

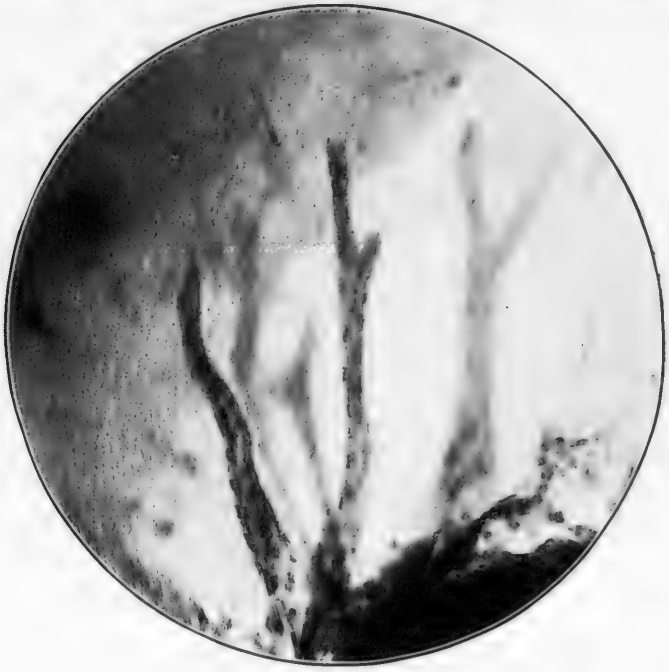


FIG. 1.

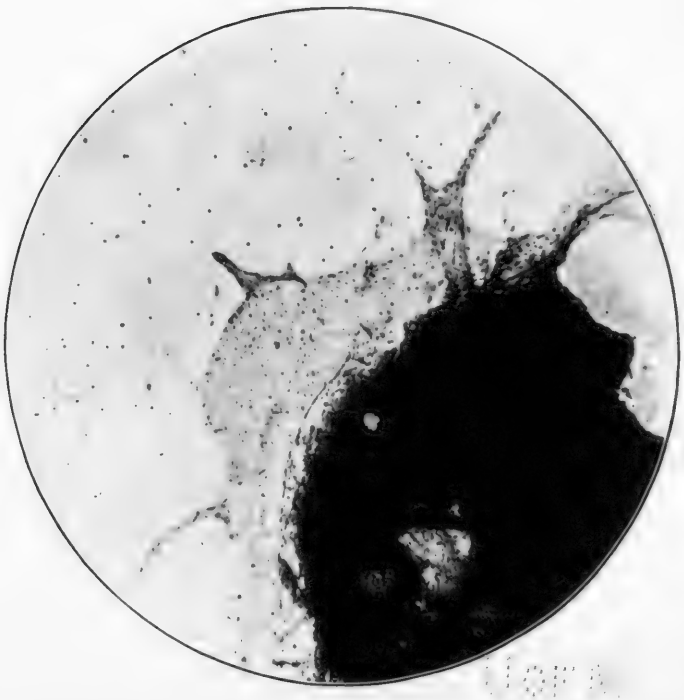


FIG. 2.

Mrau

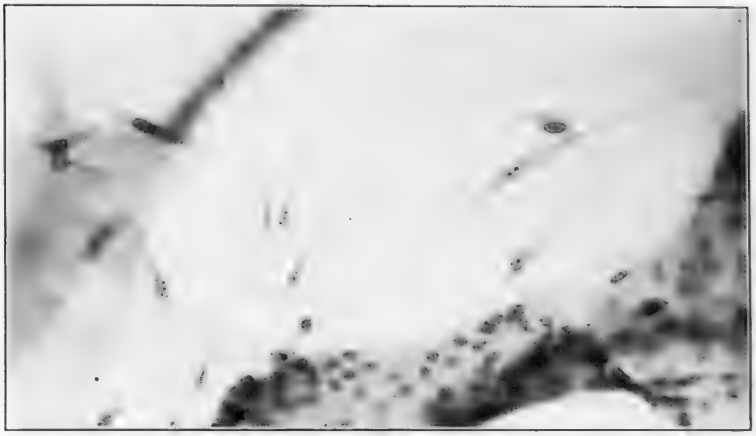


FIG. 3.

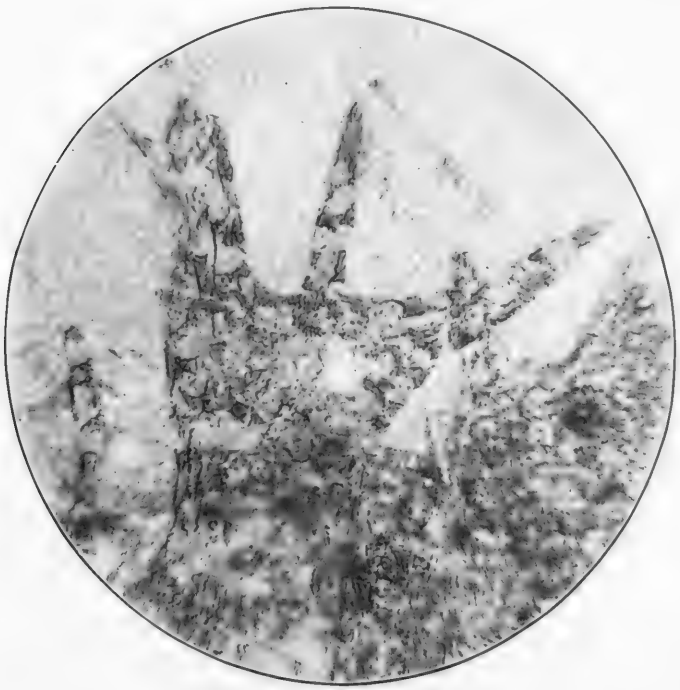


FIG. 4.

MoU

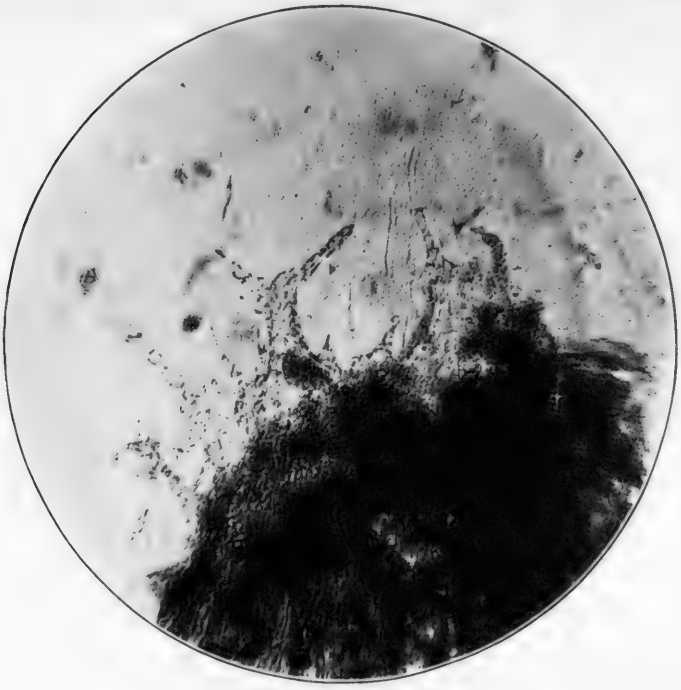


FIG. 5.

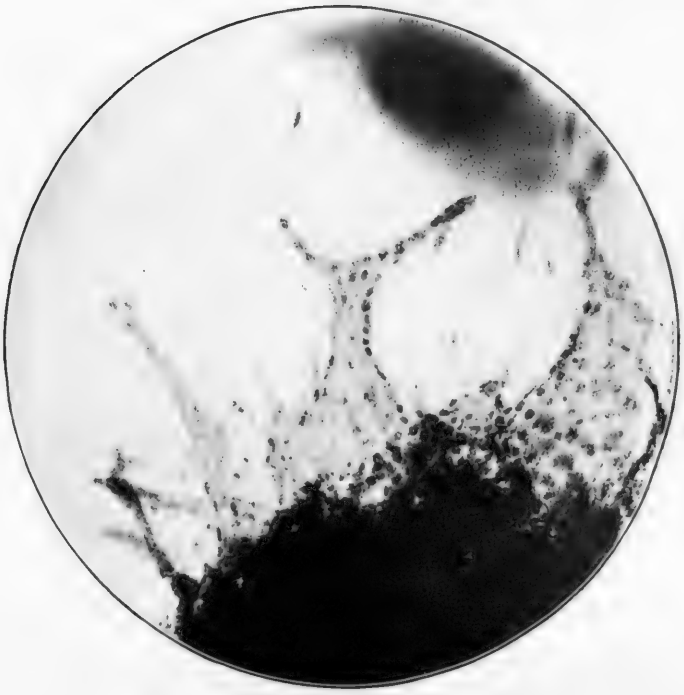


FIG. 6.

EXPLANATION OF PLATES.

PLATE LV.

FIG. 1. Tubular growth of a twelve day old secondary culture from the thyroid gland of a cat. Stain hematoxylin.

FIG. 2. A continuous layer of thyroid cells from a ninety-six hour culture, and two tubes located near the border of this layer and opening on its surface. Stain hematoxylin.

PLATE LVI.

FIG. 3. The connective tissue cells shown in a portion of Fig. 2.

FIG. 4. Cellular growth in a living culture, seventy-two hours old, of the thyroid gland of a dog.

PLATE LVII.

FIG. 5. Tubular formation from a living culture, ninety-six hours old, of the thyroid gland of a dog.

FIG. 6. The culture shown in Fig. 5, after it was fixed and stained with hematoxylin.

CICATRIZATION OF WOUNDS IN VITRO.*

By EDWARD S. RUTH.

(From the Laboratories of the Rockefeller Institute for Medical Research, New York.)

PLATES LVIII AND LIX.

For the study of the reparation of the skin and its modifications under the influence of certain substances, it became necessary to develop a method permitting observation of the cicatrization in a medium of known composition. Last October, Carrel and Burrows cultivated *in vitro* in a drop of plasma several fragments of skin of the adult frog. The present work was begun by making experiments of the same character. It was found that fragments of skin, placed less than 0.3 millimeter apart were rapidly united by an epithelial bridge. In some cases they were attracted towards each other and their edges directly united. This process is somewhat analogous to normal cicatrization.

Then I attempted, at the suggestion of Dr. Carrel, to develop a technique which would be of practical use in the study of the healing of wounds.

The method consisted of making an incision or a rectangular wound in the middle of a small fragment of skin and cultivating it outside the body in a drop of plasma (figure 15). Skin of frogs and guinea pigs was used. The skin of the guinea pig produced a very luxuriant growth of connective tissue cells, but the epidermization could not be observed easily. The skin of adult frogs generated almost exclusively epithelial cells (figure 16). Therefore it was very easy to observe the different stages of the cicatrization of the wound and to record them by camera lucida drawings.

Immediately after the preparation of the cultures, the edges of the wound appear sharply cut and the open space is free of cells (figure 1). The reparation begins very soon. It consists of three

* Received for publication, February 8, 1911.

mechanisms; the sliding of the epithelium from the edges of the wound into the open space, the epidermization, and the progressive contraction of the original edges of the wound.

The first phenomenon, the sliding of the epithelium towards the center of the wound (figure 2), occurs during the first six or twelve hours. It may be observed about the entire periphery of the wound or only a part of it. Then the second mechanism comes into play: from the edges of the shifted epithelium, epithelial cells begin to wander out either as a thin continuous layer (figure 3) or as isolated cells. This movement is rarely uniform. Very soon the opposing edges are united by a bridge of epithelial cells. They grow towards each other at the rate of about 0.06 millimeter per hour, and very soon the open space is completely covered by epithelial cells. A wound of about 0.82 by 0.32 millimeter may be completely covered by epithelium about ten hours after the preparation of the culture (figure 4). During that period, there is generally no change in the relations of the primitive edges of the wound.

When the epidermization is completed, the distance between the opposite edges of the original wound begins to grow smaller. The rate is at first very slow (figures 5, 6, 7) and at the same time the epithelium covering the wound becomes more dense (figures 8 to 13). Progressively the space between the edges diminishes and finally the epidermized surface, which may be considered as being the scar of the wound, may be less than one quarter of the original area of the wound (figure 14).

Thus the process of the healing of a wound outside the body has been observed and recorded by camera lucida drawings in five experiments. The contraction of the edges of the wound may occur before the epidermization is completed, and the wandering of the epithelial cells may also be different. But the process, in a general way, is identical with the process of cicatrization diagrammatically represented.

In conclusion, it may be stated that it is possible to produce the cicatrization of a wound *in vitro* and to observe microscopically all stages of the process.

EXPLANATION OF PLATES.

PLATE LVIII.

Camera lucida drawings diagrammatically representing the stages of cicatrization in a rectangular wound.

FIG. 1. The outline of a wound made in a piece of frog skin. The clear central area represents the portion that was completely excised. The black border represents the edges of the wound and the immediately adjacent parts of the skin.

FIG. 2. The same wound, six hours later. The parallel lined area represents the epidermis which has moved in mass into the wound. The irregular central outlined portion represents the wandering epithelial cells.

FIG. 3. The same wound, one hour and fifteen minutes later. The wandering epithelial cells have covered the entire center except for three small open areas.

FIG. 4. One hour and five minutes later the entire central portion is covered by wandering epithelial cells. There has been a slight shortening of the short diameter of the wound at the central portion.

FIGS. 5, 6, 7, 8. The central cell layer is now several cells in thickness; the epidermis, represented by parallel lines, has retracted.

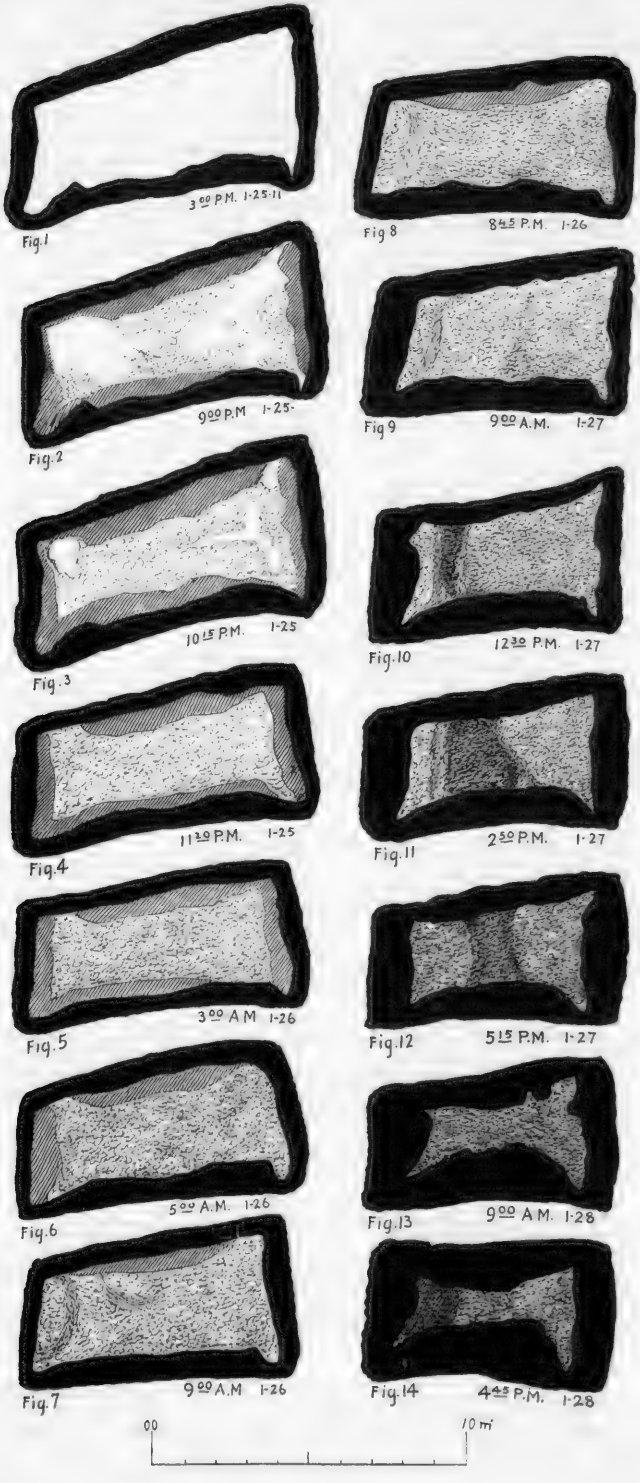
FIG. 9. The wound has shortened considerably in its long, and to some extent in its short dimension by the moving together of the connective tissue mass.

FIGS. 10, 11, 12, 13, 14. These figures show a constant increase in the thickness of the epithelial layer in the center, and a constant moving together of the mass of connective tissue which forms the border of the wound.

PLATE LIX.

FIG. 15. The dark mass is a piece of frog skin, the clear center representing the wound.

FIG. 16. The dark mass is the original piece of frog skin. The adjacent V-shaped area is the epidermis, which has moved out in mass. The extensive outer cellular area is a single layer of new-grown epithelial cells.


3 00 P.M. 1-25-11
Fig. 1
9 00 P.M 1-25-
Fig. 2
10 15 P.M. 1-25
Fig. 3
11 30 P.M. 1-25
Fig. 4
3 00 A.M 1-26
Fig. 5
5 00 A.M. 1-26
Fig. 6
9 00 A.M 1-26
Fig. 7
8 45 P.M. 1-26
Fig 8
9 00 A.M. 1-27
Fig 9
12 30 P.M. 1-27
Fig. 10
2 50 P.M. 1-27
Fig. 11
5 15 P.M. 1-27
Fig. 12
9 00 A.M. 1-28
Fig. 13
4 45 P.M. 1-28
Fig. 14
00
10 m'

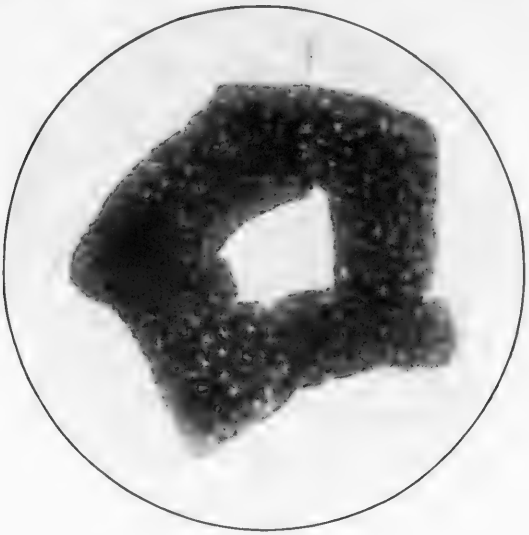


FIG. 15.

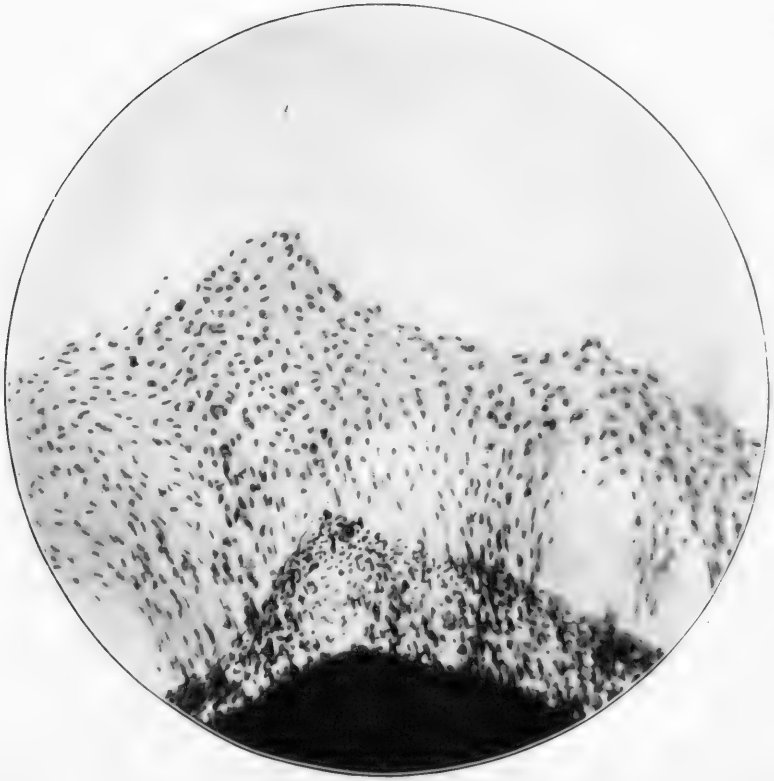


FIG. 16.

STUDIES IN ISOAGGLUTINATION.

I. TRANSFUSION AND THE QUESTION OF INTRAVASCULAR AGGLUTINATION.*

By REUBEN OTTENBERG.

(*From the Pathological Laboratory of Mount Sinai Hospital and the Laboratory of Biological Chemistry of Columbia University, College of Physicians and Surgeons, New York.*)

INTRODUCTION.

This paper offers a solution of a special problem in isoagglutination; namely, the question whether transfusion is permissible between persons whose sera agglutinate the red blood cells of each other. It also shows why such transfusions are not regularly followed by serious symptoms.

The isoagglutination of human red blood cells was discovered independently by Landsteiner and by Shattock in 1900. Many workers at first regarded the phenomenon as one of pathological significance. Halban, Ascoli, and others, however, showed that isoagglutination occurred with a large proportion of normal bloods, and Landsteiner discovered the remarkable fact that all human bloods can be divided into three sharply defined groups, according to the way in which they interagglutinate. To these groups was subsequently added a fourth, independently discovered by several observers. The groups are as follows (see table 1):

The serum of the first group, designated in this paper as group I, possesses the power of agglutinating the red cells of members of all the other groups, but the red cells of members of group I are not agglutinated by any human serum. This group includes about 50 per cent. of all persons examined.

The serum of members of the second group (group II) can agglutinate the cells of persons belonging to the third and fourth groups, but not of other members of the second group itself nor, of course, of group I. The cells of mem-

*Received for publication, February 5, 1911. This study is one of a projected series on *isoagglutination*, which in turn constitutes a section of a comprehensive plan of research on the composition of protoplasm as well as the structural and dynamic relationships of cell constituents and products. These investigations are now in progress in the Laboratory of Biological Chemistry of Columbia University, at the College of Physicians and Surgeons, and under the auspices of the George Crocker Special Research Fund.

bers of the second group can be agglutinated by sera of individuals of group I and of group III only.

The third group is the reciprocal of the second group; its serum agglutinates cells of persons belonging to members of the second and fourth groups. Its cells are agglutinated by sera of the second and fourth groups, and, of course, of the first group. Members of this group sometimes show slight individual irregularities, the cells now and then failing to be agglutinated by the sera of some members of the second group, although being agglutinated by others, and the sera occasionally agglutinating the cells of some, but not all, other members of the third group itself.

The fourth group, whose members are relatively rare, is characterized by possessing no agglutinin for human red cells and by its cells being agglutinable by the sera of all other groups.

TABLE I.

Interagglutination Reactions of Ten Individuals.

			Sera.									
Groups.			I				II	III				IV
			1	2	3	4	8	6	7	9	10	5
Cells	I	1	−	−	−	−	−	−	−	−	−	−
		2	−	−	−	−	−	−	−	−	−	−
		3	−	−	−	−	−	−	−	−	−	−
		4	−	−	−	−	−	−	−	−	−	−
	II	8	+	+	+	+	−	+	+	+	+	−
	III	6	+	+	+	+	−	−	−	−	−	−
		7	+	+	+	+	+	−	−	−	−	−
		9	+	+	+	+	+	−	−	−	−	−
		10	+	+	+	+	+	−	−	−	−	−
	IV	5	+	+	+	+	+	+	+	−	?	−

Landsteiner himself suggested that the groupings could be explained by assuming the existence of two agglutinins, of which the second group possessed one, the third group the other, the first group both, and the fourth group neither. In each case the cells are susceptible only to that agglutinin which does not exist in the individual's own serum. (Moss has, in a recent paper, made the quite unnecessary assumption that there are three agglutinins.)

The group characteristics are permanent for each individual throughout his life. When concentrated, the agglutinins act almost instantaneously; when diluted they act more slowly. They act in the cold as well as at high temperatures and they are relatively thermostable. Hektoen, who first worked out many of their characteristics, has shown that the agglutinin is absorbed by the cells which it agglutinates.

These agglutinins, like other serum agglutinins, are probably associated with the euglobulin of the blood. I have recently found that when agglutinative serum in collodion sacs is dialyzed against running water, the precipitate which forms in the serum can be redissolved in saline solution and contains the agglutinin in only slightly weakened concentration.

The peculiar groupings are not only permanent with the individual but they

are hereditary. In 1908, in a paper[1] by Dr. A. A. Epstein and the author, we stated that these agglutinins were inherited and followed the Mendelian law. At that time I had examined only five families and was unable to prove completely the assertion. Recently Von Dungern and Hirschfeld have examined seventy-two families and have conclusively proved that the agglutinins are hereditary and follow Mendel's law. From this explanation of the general nature of isoagglutination, I return to the special subject of the present paper.

Landsteiner, Hektoen, and, in fact, almost all who have written on the subject, as well as Crile in his work on transfusion, have expressed the opinion that isoagglutination might be a danger in transfusion. Nevertheless, the surgeons have performed a large number of transfusions without making any serum tests, and in most of the cases there have been no accidents. There have been accidents and untoward symptoms, however, both in published and unpublished cases.[2]

The only direct observations on the present question are those in a recent communication by W. Schultz.[3] Schultz made intravenous injections in ten cases of small amounts (5 to 250 cubic centimeters) of defibrinated blood. In his first case the serum of the patient was agglutinative as well as hemolytic toward the donor's red cells. After the injection of 50 cubic centimeters of blood there was a collapse, followed by vomiting, diarrhea, fever, and edema of the face and hands. In case 6, however, in which there was neither agglutination nor hemolysis between the two bloods, there was also a collapse after the injection of only 5 cubic centimeters of blood. In case 4 there was mutual agglutination but no hemolysis. Schultz, made cautious by the first experience, injected only 5 cubic centimeters of defibrinated blood. No ill effects followed. Shultz concluded that agglutinative blood is an absolute contra-indication to transfusion.

The numerous experiments by Flexner, Pearce, Coca, and others, demonstrating that various lesions and symptoms, including death, could be produced by the agglutinative sera or agglutinable cells of foreign species, do not bear directly on the present subject.

[1] *Tr. of the New York Path. Soc.*, 1908, viii, 117.

[2] The results of a study of transfusions with regard to agglutination and hemolysis tests made at Mt. Sinai Hospital during the past two years, will be published separately by Dr. Kaliski and the author.

[3] *Berl. klin. Wchnschr.*, 1910, xlvii, 1407.

EXPERIMENTAL PART.

I have found that the explanation of the inconsistency between the very sharp test tube agglutination, and the frequent absence of symptoms on transfusion of agglutinative blood, depends on the quantitative relations between agglutinin and agglutinable cells, and non-agglutinable cells.

While performing some experiments for the purpose of determining the bulk of agglutinable cells that is necessary for complete absorption of all the agglutinin from a given serum, I noticed that when the amount of red cells exceeded a certain ratio to the amount of serum, all the agglutinin was absorbed without, however, any agglutination taking place, so far as the naked eye could determine.

This was confirmed by several experiments, of which one only is referred to in table II. In this experiment blood cells belonging to group II were obtained in normal salt solution containing 1 per cent. of sodium citrate. The cells were washed once in salt solution, then again sedimented by centrifuging for about ten minutes at high speed. All the supernatant fluid was decanted and the concentrated cells were used in the experiment. An actual count (with Thoma-Zeiss hemocytometer) showed 10,000,000 red blood cells per cubic millimeter. It has been calculated (P. Weber) that there is only space for 13,000,000 human red cells in one cubic millimeter, so the suspension used might be designated as a 75 per cent. suspension.

The serum used was of class I. Its exact agglutinative titer was not determined, but the serum was known to be active in a dilution of at least 1 to 12. The mixtures were made in the proportions stated, kept at room temperature for thirty minutes, again thoroughly mixed, and kept in an ice box over night. On the following day the serum was pipetted off and its agglutinative power tested against a 5 per cent. suspension of the same supply of red cells.

From table II it may be seen that one volume of the cell suspension absorbed all the agglutinin from at least sixteen volumes of serum, and that as one volume of cells was still completely agglutinated by thirteen volumes of serum, the cells were able to absorb more agglutinin than was actually necessary to agglutinate them. Controls with non-agglutinable cells showed no absorption of agglutinin. On the other hand, above this concentration of one part of

cells to thirteen parts of serum, the agglutination diminished in intensity, all the agglutinin being still absorbed; and when the concentration of four volumes of cells to six volumes of serum was reached, agglutination could no longer be observed, either macroscopically or on microscopic examinations of a drop of the mixture. It was at first thought that in these concentrated cell mixtures the agglutinin was distributed among the cells in such a way that too little of the active substance went to each cell to produce the visible physical change known as agglutination. Later, however, when the work was repeated and the microscopic examinations were made in a different way, namely, by diluting the mixture with an excess of salt solution or of Hayem's fluid, it was seen that some agglutination also occurred in the more concentrated mixtures, but that the clumps were extremely small (four to five cells) and had been masked previously by the excess of densely concentrated non-agglutinated cells.

TABLE II.

The Absorption of Agglutinin by Agglutinable Cells.

Volumes.		Agglutination.	Absorption of agglutinin.
Serum.	Cells.		
4	6	−	All absorbed.
5	5	−	" "
6	4	−	" "
7	3	+ (microscopic).	" "
8	2	+ (naked eye).	" "
10	1	almost complete.	" "
13	1	complete.	" "
14	1	"	" "
15	1	"	" "
16	1	"	" "
17	1	"	Partly absorbed.
18	1	"	" "
19	1	"	Not absorbed.
20	1	"	" "
21	1	"	" "

In transfusion, however, two other substances may accompany agglutinable cells and agglutinative serum (plasma). These are non-agglutinative serum (or plasma) and non-agglutinable cells. The question therefore arises whether non-agglutinative serum exerts any hindering effect on agglutination. Hektoen states that the addition of increasing quantities of serum from one group to

sera of the other groups does not hinder agglutination of the proper corpuscles. The author has reëxamined the question by directly comparing the results of dilution with saline solution and dilution with neutral (non-agglutinative) serum (table III).

Two specimens of blood (labeled E and K) obtained by defibrination were used, the one of class II and the other of class III.[4] The cells of one (K) were washed and made up to a 5 per cent. suspension in 0.9 per cent. saline solution. Two parallel series of dilutions of the agglutinative serum E were then made; in one series saline solution was the diluent; in the other, neutral (non-agglutinative) serum K.

TABLE III.

Agglutination in Diluted and in Mixed Sera.

Proportions of			Actual dilution of active serum.	Agglutination.	
Active serum.	Diluent.	Cell suspension.		With *saline* as diluent.	With *serum* as diluent.
2	7	3	1 : 6	Complete: 2 or 3 clumps.	Complete: numerous small clumps.
1	5	2	1 : 8	Complete: 2 or 3 clumps.	Complete: numerous small clumps.
2	13	5	1 : 10	Complete: 2 or 3 clumps.	Complete : numerous small clumps.
1	8	3	1 : 12	Complete: 2 or 3 clumps.	Complete: numerous small clumps.
2	19	7	1 : 14	Complete: 2 or 3 clumps.	Complete : numerous small clumps.
1	11	4	1 : 16	Complete: 2 or 3 clumps.	Complete : numerous small clumps.
2	25	9	1 : 18	Complete: 2 or 3 clumps.	Complete : numerous small clumps.
1	14	5	1 : 20	Numerous small clumps.	Very fine clumps.
1	17	6	1 : 24	Fine clumps.	Clumps seen only through microscope.
1	20	7	1 : 28	Very fine clumps.	Clumps seen only through microscope.
1	23	8	1 : 32	Clumps visible only through microscope.	No agglutination.
1	26	9	1 : 36	No agglutination.	No agglutination.

To each test tube was added exactly the same proportion of cell suspension; namely, one-fourth of the total volume. The actual dilution of the agglutinative serum is shown in the fourth column of table III. It is seen that when saline solution was the diluent, agglutination was still observable at a dilution of 1 to 32; when

[4] It was not determined which specimen was of class III; and the determination would be of no significance in the present instance.

serum was the diluent, it occurred at a dilution of 1 to 28. There is then only an extremely slight interference, if any, on the part of neutral serum.

The qualitative differences between the two series, however, are more significant than the quantitative. Where saline solution was the diluent there were, in all the dilutions below 1 to 20, only two or three large compact clumps. Where serum was the diluent, on the other hand, there were very many small clumps.

The second question, whether the presence of non-agglutinable cells in the mixture interferes with agglutination, was investigated by making mixtures of whole defibrinated bloods in varying proportions, instead of serum and washed cells. These mixtures imitated then, as closely as can be done in a test tube, the actual conditions in transfusion (table IV).

TABLE IV.

Mixtures of an Agglutinative and an Agglutinable Whole Blood. Defibrinated Blood of Class I (Agglutinative Serum, Non-Agglutinable Cells) and of Class II (Agglutinable Cells, Non-Agglutinative Serum).

Proportions.		Agglutination.*
Blood (class II).	Blood (class I).	
1	9	+
2	8	+
3	7	+
4	6	+
5	5	+ (greatest number of clumps).
6	4	+ (smaller numer of clumps).
7	3	+ (only a few clumps).
8	2	−
9	1	−

*The clumps were all microscopic in size.

The mixed bloods were thoroughly shaken at once and again in about thirty minutes, at room temperature. They were then put on ice and observations were made at the end of twenty-four hours.

None of the mixtures exhibited macroscopic agglutination; all of them formed, when shaken, thick emulsions. Through the microscope it was seen, however, that microscopic agglutination had occurred in seven of the nine mixtures. The presence of non-agglutinable cells in the mixtures, then, did not prevent agglutina-

tion, but did change entirely the nature of the agglutination;—instead of a few large clumps, innumerable microscopic clumps were produced. This was particularly true of the first five mixtures in the series; in the other mixtures the absorption of agglutinin by the excess of agglutinable cells present also played a part; and in the last two, they apparently prevented agglutination, as in the experiments referred to in table II.

This, of course, is extremely significant for transfusion; a few large clumps might cause serious or fatal embolism. A considerable number of microscopic clumps, becoming plugged in widely scattered capillaries might cause few or no symptoms. The microscopic appearances were controlled by making a similar series of mixtures of each of the specimens of blood used with another blood of its own class. In none of these were any clumps observed.

From this experiment it is seen, then, that when the proportion of agglutinative to agglutinable blood is small (1 to 4 or less), no agglutination occurs, all the agglutinin being absorbed by the excess of substratum. When the proportion is reversed, agglutination occurs but is microscopic. When the bloods are mutually agglutinative (one of class II, the other of class III), one finds, likewise, that the blood shows no agglutination to the naked eye but shows microscopic clumps in all proportions (table V). In this instance both of the inhibitory factors are in play; either the one or the other variety of red cells being always present in too great a proportion to be agglutinated completely. The excess of cells functionates as non-agglutinable, and interferes (probably mechanically) with the agglutination of the other species of red cells, which, in many of the mixtures, are present in small amounts compared to their agglutinative serum, and would otherwise be agglutinated completely.

There is no doubt, then, that concentrated non-agglutinable cells interfere with the agglutination of actually agglutinable cells present in the same mixture. This interference is probably mechanical; it does not occur when both varieties of cells are present in small amounts.

Additional experiments were performed to determine whether similar conditions prevail when one or the other blood is very

TABLE V.

Mixtures of Mutually Agglutinative Bloods.

Proportions.		None of the mixtures showed any agglutination to the naked eye, but all did microscopically.*
Blood 8 (class III)	Blood 3 (class II).	
9	1	Numerous small compact clumps.
8	2	Small clumps of 10 to 30 cells.
7	3	A few small clumps.
6	4	"
5	5	"
4	6	"
3	7	"
2	8	"
1	9	More numerous compact clumps, 20 to 24 cells.

*The microscopic examinations were made by diluting with Hayem's fluid, in which further agglutination can not occur.

anemic, as is practically always the case in patients requiring transfusion. From the defibrinated whole bloods of normal people. artificial anemic bloods were made by pipetting off one volume of the sedimented red cells and suspending it in nine volumes of the corresponding serum. These anemic bloods then contained less than one million red cells per cubic millimeter. Mixtures were made (table VI) in such a way that in one series (A) the anemic blood was agglutinative, in the other (B) the anemic blood was agglutinable. From the results in series B it appears that, if the anemic patient's cells are agglutinable by the donor's serum, a considerable amount (at least one-fourth as much as the total blood volume of the patient) can be transfused before the danger of intravascular agglutination occurs. If, as in A, the contrary is true, and the anemic patient's blood is agglutinative toward the full-blooded donor's cells, the likelihood of intravascular agglutination is much greater and disappears only when a large amount (more than the volume of the patient's blood) is transfused. The same should be true for mutually agglutinative bloods.

This would depend, of course, to a considerable extent on the rapidity of mixing of transfused bood. The assumption need not be made that mixing occurs very rapidly, because when the hindering factors are present—dilution of agglutinative serum, excess of agglutinable cells, interference by non-agglutinable cells—any agglutination that does take place occurs very slowly.

TABLE VI.

Series A.

Mixtures of Anemic Blood No. 10 (Agglutinative, Class I) and Full Blood No. 1 (Agglutinable, Class II).

Proportions.		Agglutination.
Anemic No. 10.	Full No. 1.	
9	1	Fine clumps seen with naked eye.
8	2	"
5	5	Microscopic clumps only.
2	8	Doubtful, a few small clumps.
1	9	No clumps.

Series B.

Mixtures of Full Blood No. 10 (Agglutinative, Class I) and Anemic Blood No. 1 (Agglutinable, Class II).

Proportions.		(Microscopic clumping only.)
Full No. 10.	Anemic No. 1.	
1	9	No clumps.
2	8	No clumps.
5	5	Many sharply defined clumps.
8	2	Smaller number of clumps.
9	1	Very few clumps, but all sharply defined.

Above and beyond all the considerations presented here, there remains a possibility that the isoagglutinins are substances which, like fibrin ferment, are formed only after the blood leaves the vessels. This, though possible, seems highly improbable. The agglutinins are present no matter how the cells and serum are obtained,—whether by defibrination, ordinary clotting, citration, and the use of oxalate or hirudin. Furthermore, I have recently found them to occur in most transudates and exudates.

CLINICAL PART.

I have recently seen several transfusions which confirm the view that in the presence of an excess of agglutinable cells all the agglutinin can be absorbed without doing harm.

In the first case the patient was a young woman with a bleeding gastric ulcer. Her blood belonged to the second or third agglutinative class, while that of the donor, who was chosen in the emergency, belonged to the first; that is, the donor's serum agglutinated the patient's cells, but his cells were not agglutinated by the patient's

serum (the same instance as that in table VI, series B). Sufficient blood was transfused to carry the patient's hemoglobin from 18 at the beginning to 40 per cent. at the end of the transfusion. By calculation, this must have been about forty per cent. as much as the patient's own blood volume.[5] No untoward symptoms occurred and the patient subsequently recovered. On examining the patient's serum, obtained thirty-six hours after the transfusion, it was found to contain no agglutinin of class I; it did not agglutinate her cells obtained either before or after the transfusion. All the agglutinin of class I which had been transfused into her had disappeared.

A confirmation of the view expressed here is also furnished by a case recently described by J. G. Hopkins.[6] The transfusion was instituted for the relief of an extreme anemia resembling the primary pernicious type. The patient's serum agglutinated the donor's cells but the donor's serum did not agglutinate the patient's cells (the more dangerous instance, as shown in table VI, series A). Immediately following transfusion, smears of the peripheral blood showed large numbers of polymorphonuclear leucocytes containing red blood cells.

After the transfusion, the patient was incontinent of feces, and irrational. Six hours later he developed hemiplegia and coma. Nine hours after the transfusion he died.

"The post-mortem serum did not agglutinate the donor's corpuscles". "The agglutinins present when the serum was first examined were evidently bound". Hopkins is "unable to determine the meaning of the blood-picture in this case" and believes that "what was observed was probably phagocytosis of the transfused red cells by the phagocytes of the recipient".

We have here a case, then, in which apparently all the agglutinin in the body was absorbed by the transfused red cells. It seems at least probable that the death was due to intravascular agglutination, and that the phagocyted red cells were such as had already been

[5] Patient's hemoglobin, at beginning = 18 per cent.; at end = 40 per cent. Donor's hemoglobin, at beginning = 90 per cent. Let the relation of volume transfused to the patient's original blood volume = x : 1; then $18 + 90x = 40(x + 1)$ and $x = 22/50 = 0.44$.

[6] Hopkins, *Arch. Int. Med.*, 1910, vi, 270.

affected by the agglutinative serum and were in effect foreign bodies, although on account of the hindering factors mentioned in the present article, they had not yet become actually agglutinated.

Since the publication of Hopkins' paper I have had the opportunity of studying the blood of three transfusions with regard to phagocytosis. In the first two cases a number of candidates were examined, and a donor was chosen who belonged to the same agglutinative group as the patient. In these cases no phagocytosis of red cells was observed.[7] In the third case the surgeon elected to disregard the blood tests. The patient's serum was agglutinative toward the donor's red cells, but the donor's serum was not agglutinative to the patient's cells. The patient's serum was also very slightly hemolytic.

Blood smears made at the end of transfusion showed that many of the polynuclear leucocytes were phagocyting red cells in the peripheral circulation of the patient. The leucocytes contained from one to three red cells each. No agglutinated red cells could be seen in the smears, nor were any seen in a fresh drop of blood diluted with sodium citrate in normal salt solution.

The examination of cells and serum after the transfusion showed a remarkable condition of affairs (table VII).

TABLE VII.

P_1 = Patient's blood taken before transfusion.
P_2 = Patient's blood taken at end of transfusion.
D = Donor's blood.

		Sera.		
		P_1	P_2	D
Cells.	P_1	−	−	−
	P_2	+	−	−
	D	+	−	−

The serum after transfusion no longer agglutinated the donor's cells; as in the two cases above, the agglutinin had all been absorbed.

[7] For one of these cases I am indebted to Dr. Kaliski, with whom I shall at a later date publish more detailed accounts of all the cases referred to.

On the other hand, the patient's cells after transfusion were agglutinated markedly by his own serum obtained before transfusion, but not, of course, by his own serum after transfusion. This proves definitely that the donor's cells in the patient's circulation had not lost their agglutinability, but that most of them were not agglutinated simply because there was not enough agglutinin.

The absence of phagocytosis of red cells in two transfusions of non-agglutinable blood, and its occurrence in two transfusions of agglutinable blood, indicate clearly that there is a close connection between agglutination and phagocytosis. It further makes it probable that the transfusion of agglutinable blood, even if no accident occurs, is useless, as the agglutinable cells are foreign and do not remain in the patient's circulation.[8]

CONCLUSIONS.

1. Intravascular agglutination can occur, and is the probable cause of occasional untoward symptoms, or even death, following transfusion of agglutinative blood. In the majority of cases, however, it does not occur, or if it does, it causes no symptoms. This is dependent on the influence of three factors: (1) concentration of the agglutinin; (2) absorption of the agglutinin by an excess of agglutinable cells; (3) interference with agglutination by an excess of non-agglutinable cells, so that when clumps occur they are microscopic in size.

2. If, for a given transfusion, a non-agglutinative donor, i. e., a donor whose blood is of the same agglutinative class as the patient's, can not be obtained, then it is safer to use a person whose serum is agglutinative toward the patient's cells than one whose cells are agglutinated by the patient's serum.

3. Tests for agglutination, as well as for hemolysis, ought to be made before transfusion. When time does not permit this, one has

[8] Since writing the above, I have had an opportunity to confirm further these views in two cases. One was a transfusion between persons of the same agglutination class. No phagocytosis was seen. The other was a transfusion in which the bloods were mutually agglutinative. Extensive intravascular phagocytosis of erythrocytes occurred. Other observations on these cases will be published later.

to weigh the possible dangers of agglutination or hemolysis against the dangers of letting the patient go without transfusion.

4. Agglutinable cells when transfused are taken up by the phagocytes in the patient's blood; and, for this reason, the transfusion of agglutinable blood, even when no accident happens, can be expected to do little permanent good.

CHANGES IN NITROGENOUS METABOLISM AFTER PARATHYROIDECTOMY.*

By JEAN V. COOKE, M.D.

Resident Pathologist to the Pennsylvania Hospital, Philadelphia.
(From the Carnegie Laboratory, University and Bellevue Hospital Medical College, New York.)

That the function of the parathyroids is in some manner connected with the metabolism of protein was suggested in 1894 by the experiments of Verstraeten and Vanderlinden,[1] who observed that the tetany following parathyroidectomy was more violent and appeared sooner after the operation if the animals were kept on a meat diet. Within the past two years, MacCallum and Voegtlin,[2] in a study of dogs with parathyroid tetany, found in the urine an increase in the excretion of total nitrogen and ammonia with an increased ammonia ratio. In the blood there was noticed an increase in the amount of ammonia during tetany, and in one case lactic acid was found. Underhill and Saiki[3] found an increased ammonia percentage excretion after complete thyroparathyroidectomy, and Berkeley and Beebe[4] state that they have confirmed this.

Later Underhill and Hildilch[5] found that this increased percentage of ammonia in the urine was not present after thyroidectomy alone. Musser and Goodman,[6] in a study of a case of tetany following removal of the thyroid and presumably the parathyroids, report a greatly increased excretion in urinary ammonia. In addition to the foregoing observations on metabolism, Thompson and

* Aided by a grant from the Rockefeller Institute for Medical Research. Received for publication, January 20, 1911.

[1] *Mém. couron. Acad. roy. de méd. de Belg.*, 1894, xiii, 1.

[2] *Jour. Exper. Med.*, 1909, xi, 118.

[3] *Jour. Biol. Chem.*, 1908, v, 225.

[4] *Jour. Med. Research*, 1909, xx, 149.

[5] *Am. Jour. Physiol.*, 1909–10, xxv, 66.

[6] *Univ. Penn. Med. Bull.*, 1909–10, xxii, 85.

Leighton[7] found that the slow destruction of parathyroid tissue brought about by ligating the glands is followed by a chronic nutritional disturbance with progressive loss of weight and strength, and a final stuporous condition ending in death without tetany. This same condition has been noticed by other investigators in animals from which presumably all parathyroid tissue had been removed. It is usually considered that some remnant of parathyroid which remained imbedded in one of the thyroid lobes was sufficient to prevent an acute insufficiency, and that the symptoms of disordered nutrition were probably due to a chronic parathyroid insufficiency.

The experiments here reported consist of a series of observations on the urine of parathyroidectomized dogs. Various nitrogenous substances were estimated daily, and in two experiments a routine test was made for lactic acid.

The animals used were female dogs, varying in weight from 5 to 10.6 kilograms. They were kept in metabolism cages under constant and comparable conditions. All were given water daily by the stomach tube and were catheterized each day at the same hour, in order to obtain constant amounts of urine. None developed cystitis. All but one of the animals were allowed to fast during the experiments in order that the nitrogenous equilibrium would not be disturbed by anorexia or vomiting when tetany developed. All operative procedures were carried out under complete ether anesthesia.

Chemical Methods.—All estimations except those of lactic acid were done in duplicate. The methods used were as follows:

Total nitrogen	Kjeldahl's method.
Ammonia	Folin's method.
Urea	Levene and Meyer's method.[8]
Creatinin	Folin's method.
Amino nitrogen	Van Slyke's method.[9]

Lactic acid was estimated as follows: About half the daily output of urine was evaporated to dryness on the water-bath after being made slightly alkaline with sodium carbonate; the residue was extracted with alcohol, filtered, acidified with phosphoric acid,

[7] *Jour. Med. Research*, 1908, xix, 121.
[8] *Jour. Am. Chem. Soc.*, 1909, xxxi, 717.
[9] *Proc. Soc. Exper. Biol. and Med.*, 1910, vii, 46.

TABLE I.

Control Experiment.

Dog 4, black terrier. Weight 7.3 kilos. Fed constant diet beginning November 27. Allowed to fast beginning December 7. Given 350 c.c. of water daily by stomach tube.

Date.	Amount in c.c.; specific gravity; reaction.	Total N in gms.	N in form of ammonia in gms.	Per cent. of N in form of ammonia.
Dec. 2–3	450 1015 acid	5.23	0.260	5.0
" 3–4	420 1016 acid	5.14	0.254	4.9
" 4–5	440 1014 acid	5.15	0.233	4.5
" 5–6	420 1015 acid	5.32	.0286	5.4
" 6–7	205 1033 acid	5.66	0.277	4.9
" 7–8	340 1010 acid	2.76	0.146	5.4
" 8–9	460 1009 acid	2.44	0.129	5.3
" 9–10	445 1010 acid	2.40	0.114	4.7
" 10–11	380 1012 acid	2.64	0.123	4.6
" 11–12	500 1015 amphoteric	2.44	0.150	6.1
" 12–13	345 1011 acid	2.27	0.120	5.3
" 13–14	340 1011 acid	2.90	0.132	4.6
" 14–15	360 1010 acid	2.60	0.143	5.5
" 15–16	390 1009 acid	2.36	0.149	6.3
" 16–17	360 1009 acid	2.23	0.132	5.9
" 17.	Observation stopped. There is no increased excretion of nitrogen or ammonia.			

TABLE II.

Control Experiment.

Dog 9. Weight 7.8 kilos. Fed constant diet March 14 to 23. Allowed to fast March 24 to April 1. Given 300 c.c. of water daily by stomach tube.

Date.	Amount in c.c.; specific gravity; reaction.	Total N in gms.	N in form of ammonia in gms.	Per cent. of N in form of ammonia.	Remarks.
March 22–23	460 1020 acid	5.50	0.257	4.7	
" 23–24	450 1020 acid	5.46	0.275	5.0	
" 24–25	280 1010 acid	3.05	0.134	4.4	Fasting.
" 25–26	340 1009 acid	2.85	0.146	5.1	
" 26–27	290 1010 acid	2.91	0.154	5.3	
" 27–28	320 1009 acid	3.18	0.163	5.1	Operation.
" 28–29	225 1019 acid	3.17	0.183	5.8	
" 29–30	315 1010 acid	3.04	0.186	6.1	
" 30–31	230 1015 acid	3.07	0.143	4.7	
" 31–April 1	215 1015 acid	3.10	0.150	4.8	

On March 27, parathyroidectomy was performed, which was apparently incomplete. The animal developed no signs of tetany during the month following the operation. No increased excretion of nitrogen or ammonia occurred.

again evaporated, and extracted with ether. The ethereal extract was filtered, evaporated, and the residue taken up with water. If Uffelmann's test was given by this aqueous solution, lactic acid was considered to be present. To make more certain that the substance present was lactic acid, the final aqueous extracts of four successive specimens which gave Uffelmann's test were united, warmed with lead hydroxide, filtered while hot, acidified, and the lead precipitated by hydrogen sulphide. The zinc salt was then formed by heating with zinc carbonate, the solution filtered, concentrated to small vol-

ume, and the salt allowed to crystallize at room temperature. Characteristic crystals of zinc lactate were formed.

Tables I to IX show in detail the experiments and the daily urinary findings.

TABLE III.

Dog 2. Weight 9.4 kilos. Fed constant diet November 13 to 26. Allowed to fast beginning November 27. Given 350 c.c. of water daily by stomach tube.

Date.	Amount in c.c.; specific gravity; reaction.	Total N in gms.	N in form of ammonia in gms.	Per cent. of N in form of ammonia.	N in form of creatinin in gms.	Remarks.
	405					
Nov. 25–26	1023 acid	5.76				
	540					
" 26–27	1014 acid	5.74				
	360					
" 27–28	1010 acid	2.60	0.134	5.2	0.0592	Fasting.
	425					
" 28–29	1009 acid	2.32	0.123	5.3	0.0565	
	285					
" 29–30	1012 acid	2.57	0.134	5.2	0.0586	
	405					
" 30–Dec. 1	1010 acid	2.56	0.132	5.2	0.0586	
	410					
Dec. 1–2	1010 acid	2.35	0.123	5.2	0.0565	
	260					
" 2–3	1016 acid	3.08	0.160	5.2	0.0604	Operation.
	465					
" 3–4	1009 acid	3.07	0.272	8.8	0.0592	
	425					
" 4–5	1020 alkaline	5.49	0.795	14.4	0.0565	Tetany.
	255					
" 5*	1012 alkaline	1.83*	0.504	27.5	0.0227	Tetany.

On December 2, the right lobe of the thyroid and two parathyroids from the left lobe were removed. Violent tetany developed on December 4 and continued on December 5. The dog was found dead on the morning of December 6. There was a marked increase in excretion of total nitrogen and ammonia after the operation.

*The dog died before this 24 hour specimen was complete, and the urine obtained represented approximately 8 hours. The increase in the ammonia ratio, however, is quite marked.

TABLE IV.

Dog 10. Weight 6.8 kilos. Fed constant diet April 1 to 13. Allowed to fast beginning April 14. Given 250 c.c. of water daily by stomach tube.

Date.	Amount in c.c.; specific gravity; reaction.	Total N in gms.	N in form of ammonia in gms.	Per cent. of N in form of ammonia.	Remarks.
April 12–13	370 1020 acid	5.08	0.157	3.0	
" 13–14	215 1027 acid	4.93	0.154	3.1	
" 14–15	275 1011 acid	2.65	0.143	5.4	Fasting.
" 15–16	250 1010 acid	2.48	0.132	5.3	
" 16–17	235 1010 acid	1.90	0.098	5.1	
" 17–18	295 1009 acid	2.10	0.114	5.4	
" 18–19	110 1031 alkaline	2.02	0.134	6.6	Operation.
" 19–20	305 1015 acid	4.13	0.203	4.9	
" 20–21	200 1020 acid	3.50	0.207	5.9	Tetany.
" 21–22	275 1014 acid	2.87	0.172	6.0	Tetany.
" 22–23	215 1011 acid	2.29	0.143	6.2	Tetany.
" 23–24	240 1012 amphoteric	2.27	0.137	6.0	Tetany.
" 24–25	215 1011 acid	1.69	0.137	8.1	Tetany.
" 25–26	190 1011 acid	2.07	0.123	5.9	Tetany.
" 26–27	235 1012 acid	2.00	0.129	6.4	Tetany.
" 27–28	190 1012 acid	1.94	0.123	6.3	Tetany.
" 28 died					

On April 18, parathyroidectomy was performed, the entire right lobe of the thyroid and two parathyroids from the left lobe being removed. Tetany devel-

oped on April 20 and continued until the death of the animal on April 28. There is a slight but definite increase in the excretion of nitrogen and ammonia following the operation.

TABLE V.

Dog 6. Weight 5.6 kilos. Fed constant diet January 1 to 18. Allowed to fast January 19 to 27. Given 350 c.c. of water by stomach tube daily.

Date.	Amount in c.c.; specific gravity; reaction.	Total N in gms.	N in form of ammonia in gms.; per cent.	N in form of urea in gms.; per cent.	N in form of creatinin in gms.; per cent.	N in form of NH_2 in gms.; per cent.	Undetermined N in gms.; per cent.	Remarks.
	350							
Jan. 18–19	1029	5.25	0.315	4.56	0.064	0.053	0.258	
	acid		6.0	86.9	1.2	1.0	4.9	
" 19–20	lost							Fasting.
	380							
" 20–21	1010	2.96	0.189	2.52	0.055	0.053	0.143	
	acid		6.4	85.1	1.8	1.8	4.8	
	255							
" 21–22	1020	2.97	0.183	2.55	0.055	0.060	0.122	
	acid		6.2	85.8	1.9	2.0	4.1	
	350							
" 22–23	1012	3.55	0.325	2.86	0.060	0.051	0.254	Operation
	acid		9.2	80.6	1.7	1.4	7.1	
	370							
" 23–24	1011	3.40	0.457	2.60	0.054	0.061	0.228	Tetany.
	alkaline		13.4	76.5	1.6	1.8	6.7	
	380							
" 24–25	1020	6.26	0.462	5.25	0.052	0.062	0.434	Tetany.
	alkaline		7.4	83.9	0.8	1.0	6.9	
	300							
" 25–26	1019	4.93	0.589	3.90	0.055	0.068	0.318	Tetany.
	acid		11.9	79.0	1.1	1.4	6.5	
	270							
" 26–27	1022	5.62	0.478	4.62	0.062	0.074	0.386	Tetany.
	acid		8.5	82.2	1.1	1.3	6.9	
" 27 died								

Two parathyroids from the left lobe and the entire right lobe of the thyroid were removed on the morning of January 22. Tetany developed two days later and was present each following day until January 27, when the animal was found dead. After operation there was a marked increase in excretion of total nitrogen and ammonia with increased ammonia ratio and increase in undetermined nitrogen. Creatinin and amino nitrogen remained unaltered.

Dog 3 (table IX) presented some very interesting points. The animal was fed on a constant diet until practically in equilibrium, and on the morning of November 30, 1909, two parathyroids were removed from each side. Following the operation there were no signs of tetany and the animal was apparently perfectly normal. There was no change in the daily excretion of nitrogen, but for

TABLE VI.

Dog 11. Weight 6 kilos. Fed constant diet April 12 to 25. Allowed to fast April 26 to May 6. Given 300 c.c. of water daily by stomach tube.

Date	Amount in c.c.; specific gravity; reaction.	Total N in gms.	N in form of ammonia in gms.; per cent.	N in form of urea in gms.; per cent.	N in form of creatinin in gms.; per cent.	N in form of NH_2 in gms.; per cent.	Undetermined N in gms. per cent.	Lactic acid.	Remarks.
	300								
April 26–27	1010	1.99	0.143	1.73	0.046	0.052	0.019	None	Fasting.
	acid		7.2	86.9	2.3	2.6	1.0		
	270								
" 27–28	1010	2.16	0.134	1.86	0.047	0.051	0.068	None	
	acid		6.2	86.1	2.2	2.3	3.2		
	210								
" 28–29	1010	2.04	0.140	1.72	0.048	0.054	0.078	None	
	acid		6.9	84.3	2.4	2.6	3.8		
	175								
" 29–30	1015	2.52	0.149	2.09	0.045	0.053	0.183	None	Operation.
	acid		5.9	82.9	1.8	2.1	7.3		
	305								
" 30–May 1	1007	2.45	0.166	2.03	0.047	0.053	0.154	None	
	acid		6.8	82.8	1.9	2.2	6.3		
	165								
May 1–2	1024	3.63	0.249	2.93	0.053	0.084	0.314	Present	Tetany.
	acid		6.9	80.7	1.5	2.3	8.6		
	260								
" 2–3	1020	3.80	0.280	3.12	0.058	0.079	0.263	Present	Tetany.
	acid		7.4	82.1	1.5	2.1	6.9		
	280								
" 3–4	1016	3.25	0.260	2.53	0.051	0.106	0.303	Present	Tetany.
	acid		8.0	77.8	1.6	3.3	9.3		
	150								
" 4–5	1030	2.97	0.280	2.26	0.045	0.121	0.264	Present	Tetany.
	acid		9.4	76.1	1.5	4.1	8.9		
May 6 animal found dead									

The entire right lobe of the thyroid and two large parathyroids from the left lobe were removed on the morning of April 29. The dog developed tetany on the second day following, which persisted until the animal was found dead on May 6. There was an increased excretion of total nitrogen and ammonia with an increased amount of undetermined nitrogen. No change in creatinin elimination occurred and there was only a slight increase in amino nitrogen after the operation. Lactic acid appeared in the urine the day tetany was first noticed and persisted until the animal died.

four days the ammonia elimination was practically doubled. A second operation was performed on December 8, the entire left lobe of the thyroid being removed, with no alteration in the daily nitrogenous excretion, but with the same increase in urinary am-

TABLE VII.

Dog 15. Weight 10.6 kilos. Fed constant diet beginning April 19. Allowed to fast beginning April 28. Given 350 c.c. of water daily by stomach tube.

Date.	Amount in c.c.; specific gravity; reaction.	Total N in gms.	N in form of ammonia in gms.	Per cent. of N in form of ammonia.	Lactic acid.	Remarks.
April 26–27	420 1022 acid	5.27	0.247	4.7	None	
" 27–28	350 1030 acid	5.49	0.275	5.0	None	
" 28–29	370 1013 acid	3.57	0.178	5.0	None	Fasting.
" 29–30	400 1014 acid	3.27	0.185	5.6	None	
" 30–May 1	415 1011 acid	3.42	0.169	4.9	None	
May 1–2	370 1015 acid	3.11	0.160	5.1	None	
" 2–3	375 1016 acid	3.18	0.224	6.1	None	Operation.
" 3–4	270 1027 acid	5.90	0.572	9.7	Present	Tetany.
" 4–5	320 1021 acid	5.13	0.708	13.8	Present	Tetany.
" 5–6	230 1025 alkaline	5.67	0.668	11.8	Present	Tetany.
" 6–7	210 1025 alkaline	5.24	0.686	13.1	Present	Tetany.
" 7 died						

On May 2, the entire right lobe of the thyroid and two parathyroids from the left lobe were removed. Tetany developed on May 4 with increased urinary nitrogen and ammonia and the appearance of lactic acid in the urine, all of which lasted until the death of the animal on May 7.

monia. The condition of the animal remained unchanged. On December 15, the dog was put under ether anesthesia for one and one half hours to determine whether this increased ammonia excretion was due to the anesthetic. Following the anesthesia the nitrogen and ammonia remained practically unchanged, and the metabolic studies were discontinued. It was observed, however,

TABLE VIII.

Dog 1. Weight 5 kilos. Fed constant diet November 1 to 8. Allowed to fast beginning November 9. Given 250 c.c. of water daily by stomach tube, beginning November 11.

Date.	Amount in c.c.; specific gravity; reaction.	Total N in gms.	N in form of ammonia in gms.	Per cent. of N in form of ammonia.	Remarks.
Nov. 10–11	60 1049 acid	2.75	0.134	4.9	Fasting.
" 11–12	260 1014 acid	2.27	0.120	5.3	
" 12–13	110 1030 acid	2.23	0.123	5.5	
" 13–14	230 1015 acid	2.23	0.132	5.9	
" 14–15	200 1013 acid	2.10	0.123	5.9	
" 15–16	170 1021 acid	2.65	0.180	6.8	Operation.
" 16–17	215 1016 amphoteric	2.56	0.337	13.2	
" 17–18	210 1017 alkaline	2.71	0.308	11.3	
" 18–19	200 1017 amphoteric	3.27	0.280	8.6	
" 19–20	225 1022 alkaline	4.58	0.443	9.7	
Nov. 20 died.					

The left lobe of the thyroid and three parathyroids from the right lobe were removed on November 15. No tetany developed, but the dog became dull and apathetic, sleeping most of the time until she died on November 20. At autopsy, there was no infection of the operative wound and no cause for death could be found. No parathyroid tissue could be found in serial sections of the remaining right lobe of the thyroid. There was an increased elimination of ammonia and total nitrogen following the operation, although no tetany was noticed.

that the reflexes were much increased, the faintest touch of a camel's hair brush to the hair on the neck, back, and flanks causing an immediate reflex twitching of the skin, and a very light tap on the leg muscles causing an immediate contraction of the entire leg. These increased reflexes were much more

TABLE IX.

Dog 3. Weight 5.6 kilos. Fed constant diet beginning November 22. Given 300 c.c. of water daily by stomach tube.

Date.	Amount in c.c.; specific gravity; reaction.	Total N in gms.	N in form of ammonia in gms.	Per cent. of N in form of ammonia.	Remarks.
Nov. 26–27	440 1014 acid	4.32	0.225	5.2	
" 27–28	390 1015 acid	4.58	0.236	5.2	
" 28–29	320 1019 acid	4.66	0.232	5.0	
" 29–30	370 1017 acid	4.74	0.240	5.1	
" 30–Dec. 1	270 1020 acid	4.97	0.243	4.9	1st operation.
Dec. 1–2	415 1013 acid	4.41	0.366	7.6	
" 2–3	395 1018 acid	4.36	0.498	11.2	
" 3–4	350 1017 acid	4.35	0.506	11.6	
" 4–5	320 1018 acid	4.10	0.449	10.9	
" 5–6	285 1020 acid	4.29	0.297	6.9	
" 6–7	515 1020 acid	4.36	0.130	3.0	
" 7–8	265 1021 acid	4.33	0.177	4.1	
" 8–9	250 1025 acid	4.49	0.586	10.8	2d operation.
" 9–10	365 1019 acid	4.31	0.625	14.5	
" 10–11	370 1019 acid	4.13	0.423	10.2	
" 11–12	375 1022 acid	4.25	0.263	6.2	
" 12–13	330 1023 acid	4.33	0.306	7.1	

Date.	Amount in c.c.; specific gravity; reaction.	Total N in gms.	N in form of ammonia in gms.	Per cent. of N in form of ammonia.	Remarks.
Dec. 13–14	400 1020 acid	4.48	0.263	5.9	
" 14–15	305 1025 acid	4.22	0.127	3.0	
" 15–16	220 1031 acid	4.38	0.286	6.5	Anesthetized.
" 16–17	290 1028 acid	4.55	0.297	6.5	
" 17–18	415 1020 acid	4.60	0.243	5.3	
" 18–19	380 1020 acid	4.48	0.246	5.5	
" 19–20	340 1025 acid	4.46	0.243	5.4	
" 20–21	450 1018 acid	4.39	0.184	4.2	
" 21–22	390 1022 acid	4.33	0.194	4.5	
" 22–23	380 1020 acid	4.29	0.189	4.2	

marked when the animal was fed on a meat diet than when it was fed on a diet containing only a small amount of protein. Also, it was noted that when fed a liberal meat diet there could be seen twitchings of the muscles of the tongue which disappeared when the meat diet was discontinued. These phenomena persisted for six months following the operation, during which time the animal was under constant observation. On October 19, 1910, more than ten months after the operations, the animal became suddenly violently ill with vomiting, attacks of extreme dyspnœa, twitchings of the muscles, and convulsive attacks. These symptoms persisted with slight remissions until the animal died on October 23, 1910. No metabolic observations were made at this time. At autopsy no lesions were found. Serial sections of the remaining right lobe of the thyroid gland showed a hyperplasia of thyroid acini. In one portion, a very small parathyroid nodule about one millimeter in

diameter was found lying in a connective tissue trabecula just beneath the surface. On the surface of the gland at this point could be seen a portion of silk suture which was used to ligate a parathyroid at the operation.

The apparent explanation of these phenomena is as follows: After the first operation, on December 1, the animal developed a relative parathyroid insufficiency which was shown by a transient increase in the urinary ammonia. This was soon overcome, probably by hyperplasia of parathyroid tissue which remained. After the second operation, on December 9, a similar transient disturbance resulted, again followed by apparent recovery. The anesthesia on December 16 served as a control and had apparently no effect on the animal in any way. During the following ten months the animal had merely a very small nodule of parathyroid—apparently just enough parathyroid tissue to prevent any acute symptoms, and the increased reflexes after a full protein diet indicated that the dog was possibly on the verge of an acute parathyroid insufficiency. From some unknown cause the small remaining nodule of parathyroid suddenly became unable to meet the demands made upon it and the animal succumbed to the functional derangement thus created. It is interesting that such an extremely small amount of tissue could preserve the function of the parathyroid for so long a time with such few manifestations of insufficiency until the sudden fatal attack. A control experiment in which probably a much larger amount of parathyroid was left is shown in table II. This animal had no symptoms nor any urinary changes.

It will be seen from these experiments that there is after complete parathyroidectomy a marked and constant increase in the urinary nitrogen and ammonia with an increased ammonia ratio; that the creatinin and amino acids remain practically unaltered, with possibly a slight increase in the undetermined nitrogen; and that the increase in the ammonia and undetermined nitrogen is at the expense of the urea fraction. In two of the animals—the only ones which were studied in this connection—lactic acid appeared in the urine coincident with the tetany and with the increased elimination of nitrogen and ammonia. One animal, in which no tetany was noted, died with severe nutritional disturbances following the

operation, and the urine showed the same increase in nitrogen and in the ammonia fraction that characterized the dogs with severe tetany. In addition, one dog showed a marked but transient increase in the ammonia elimination after removal of practically all parathyroid tissue, and although it showed no manifestation of tetany in the meantime, except increased muscular irritability, it died ten months after the operation, after having shown typical tetanic symptoms.

That the tetany, which very frequently accompanies the complete removal of the parathyroids, is a symptom which in rare instances may not be present is shown by one of the experiments (dog 1, table VIII). Although observed closely and constantly, this animal showed no tetany, and it is scarcely possible that attacks should have intervened during the night and should have been so constantly absent in the frequent periods of observation during the day. The urinary and post-mortem findings were quite similar to those of animals which developed typical tetany, and the ammonia ratio reached on one day 13.7 per cent.

Probably one of the most significant observations in these experiments is the appearance of lactic acid in the urine after complete removal of the parathyroids, and this finding suggests that there may be other acid radicals in the urine in this condition which are less easy to demonstrate. Several explanations may be given for the presence of lactic acid in the urine: first, its excretion may depend on an increased formation by the muscles due to their increased activity in tetany; second, there may be some impairment of hepatic function, on the hypothesis[10] that one of the functions of the normal liver is the conversion of the lactic acid formed in the muscles into some other substance; third, since in this condition there is also found an increased amount of ammonia in the circulating blood,[11] the lactic acid may be formed to neutralize to a certain extent this base; fourth, its appearance may be due to a condition of diminished oxidation; or fifth, to its non-transformation into dextrose.[12]

[10] Salaskin and Zaleski, *Ztschr. f. physiol. Chem.*, 1900, xxix, 517.

[11] MacCallum and Voegtlin, *loc. cit.* Carlson and Jacobson, *Proc. Soc. Exper. Biol. and Med.*, 1910, vii, 50; *Am. Jour. Physiol.*, 1910, xxv, 403.

[12] Neubauer, *Deutsch. Arch. f. klin. Med.*, 1909, xcv, 211.

Concerning the first two hypotheses mentioned, little can be added to the statement that either may be an etiological factor in the appearance of lactic acid in the urine after parathyroidectomy. That lactic acid is formed in the body to neutralize an excess of circulating ammonia, however, seems less likely than the reverse; namely, that the ammonia is formed to neutralize an excessive formation of lactic and possibly other acids. The presence, also, in this condition of an increased urinary content of magnesium, as shown in the previous paper,[13] strengthens this view, since it would indicate that other bases in the urine besides the ammonia may be increased, as a result of excessive acid elimination. In this respect we have analogies in other conditions characterized by the presence of acids in the urine; for example, it is generally held that in diabetes the increased ammonia and alkali excretion is secondary to an excessive acid production.

Lactic acid, it is stated, appears in the urine in conditions of diminished oxidation and this may explain its presence in the urine after parathyroidectomy. A diminished oxygen consumption by the tissues is usually considered also to be accompanied by increased protein catabolism and by an increase in the urinary ammonia, and both these phenomena are found in parathyroidectomized animals. The dyspnœa too, which is such a common symptom in parathyroidectomized dogs, may be an indication of a deficient oxygenation, or an attempt to overcome it. Other conditions in which increased nitrogen elimination is found, such as diabetes, cachexia, metallic poisoning, etc., can be excluded, and it seems quite possible that the increased catabolism and lactic acid excretion is here due to an inability on the part of the body either to oxidize properly the products of intermediary metabolism, or to transform them, e. g., lactic acid, into other compounds, e. g., dextrose.[14]

To whatever cause, however, this increased elimination of lactic acid may be due, there does not seem to be any evidence that the serious condition of the parathyroidectomized animal is due to the acid itself. It seems that the augmented acid excretion is a secondary and possibly a relatively unimportant phenomenon attending a

[13] *Jour. Exper. Med.*, 1910, xii, 45.
[14] Neubauer, *loc. cit.*

profound metabolic disturbance, the nature of which it is not yet possible to determine. There is some indication that this disturbance is closely connected with the processes of oxidation. It is conceivable that an augmented excretion of alkalies and alkaline earths caused by an excess of circulating acid may have some relation to the tetany by altering the salt balance of the nerve cells with a consequent hyperexcitability of the central nervous system. This sequence of events, however, or at least the nervous hyperexcitability, does not occur in other conditions of increased acid elimination.

It is interesting to note that the creatinin excretion does not vary and that there appears to be no deficient desamidation.

FURTHER OBSERVATIONS AND EXPERIMENTS ON THE SO-CALLED THYROID CARCINOMA OF THE BROOK TROUT (SALVELINUS FONTINALIS) AND ITS RELATION TO ENDEMIC GOITRE.*

By DAVID MARINE, M.D., and C. H. LENHART, M.D.

(From the H. K. Cushing Laboratory of Experimental Medicine, Western Reserve University, Cleveland, in conjunction with the Pennsylvania State Fish Commission.)

Plates LX and LXI.

PART I.

INTRODUCTION.

In our first report[1] on this subject the following observations were emphasized:

(1) That all the trout, irrespective of age or location, in the ponds of the hatchery were affected with thyroid hyperplasia. (2) That it was most marked in the younger fish. (3) That a definite relation existed between the amount of water, the concentration of fish, and the quantity of food, and the severity of the disease. (4) That older trout are less susceptible to the disease, as shown by a tendency towards spontaneous recovery under constant conditions as regards the concentration of fish, the water supply, and the food. (5) That recovery from the less marked degrees of thyroid hyperplasia constantly occurred either on taking them from the ponds and putting them in their native environment (the brook) or on leaving them in the theoretically polluted pond water, but reducing the food and the concentration of fish, or on the addition of small amounts of iodin. (6) That our attempts to transplant the hyperplastic thyroid tissue from one fish to another of the same age and stock were negative. (7) That anatomical studies alone were insufficient criteria upon which to make a diagnosis of cancer, because the thyroid of the bony fish is normally a widely and loosely distributed non-encapsulated

* Received for publication, February 6, 1911.

[1] *Jour. Exper. Med.*, 1910, xii, 311.

gland, any growth of which would give the appearance of invasion of the surrounding tissues. (8) That there was no experimental basis for considering the disease, in its early stages at least, other than severe endemic goitre.

In the following report we shall present our observations on the earliest manifestations of thyroid hyperplasia in the very young fish; our observations on the spontaneous recovery of the affected fish when transferred to a natural environment; and our experiments with the late stages of the disease, or those stages associated with large, visible and ulcerated goitres.

These observations and experiments were made during the summer of 1910 at the same hatchery at which the earlier work was done.

OBSERVATIONS ON THE HISTOLOGICAL CONDITION OF THE THYROIDS OF TROUT FRY.

In order to determine how early in the life of hatchery trout thyroid hyperplasia (goitre) begins, we collected weekly specimens of the fry, beginning as soon as the egg membranes were shed (January 15, 1910) and continuing until October 8, 1910.[2]

Sections have been prepared from the thyroid areas of the entire series. It is difficult to distinguish the thyroid tissue in these fry up to the end of the second month, and we should hesitate to state that the thyroids at this age were hyperplastic, although there is no colloid present and the epithelium lining the follicles is high cubical. In fortunate sections it is possible to distinguish an alveolar lumen. Beginning with sections taken on March 16, one

[2] The entire series was collected from a single cypress hatch trough. This was one of a series of eight—all of the same size (13 feet long, by 20 inches wide, by 6 inches deep). Water was supplied directly from the spring in sufficient quantity to replace the contents of the trough every twenty minutes. These troughs were cleaned every day. The fry were fed four times daily for the first month with an aqueous suspension of hog's liver strained through muslin. During the second and third months, a similar suspension strained through coarser muslin was fed. Beginning with the fourth month, they were fed finely ground hog's heart muscle freed from adventitious tissues. As the fish grew, the heart muscle was ground coarser. No estimation was made of the number of fry in the trough at a given time. They were thinned from time to time and though always crowded had free swimming space.

can state positively that hyperplasia has begun. The thyroid tissue in most cases fills the areolar space about the ventral aorta. The epithelium lining the follicles is high cubical, regular in type, with bluish tinted cytoplasm. There is no stainable colloid. The first evidence of infiltration or invasion of the surrounding structures is seen in the specimens taken on April 30. The thyroid follicles have filled the aortic space and have extended around the bundles of lateral muscles and dorsally toward the floor of the pharynx. From this age (three and one half months) the growth is progressive, extending into the more resistant structures, as striped muscle and bone. As the gland follicles grow into the pharyngeal submucosa, the vascularity of the newly formed thyroid tissue is transmitted through the translucent mucosa of the pharynx as a pale red discoloration of the floor of the pharynx between the first and third gill arches. This pharyngeal discoloration was detectable in these particular fry about the middle of July (six months old). It may occur earlier in fish kept under less favorable conditions. The appearance of visible goitres is always a late manifestation, and we have never observed it until the subpharyngeal tissues were filled with thyroid tissue. Gaylord[3] has observed visible goitres in brook trout fry of about six months old. We have seen goitres in brook trout fry of about seven months old. This is not common, however, and indicates unusually favorable conditions for thyroid growth and hence unfavorable conditions of food and environment.

The results of this study show that thyroid hyperplasia begins at a very early age and is detectable at the end of the second month of extra-oval life. These fry developed thyroid hyperplasia while living in unused water direct from the spring and under unusually clean surroundings as regards any gross appearance of filth.

The crowding and the food, therefore, seem to be the most important factors, and the food would appear to be more important than the crowding. The influence that crowding may have on the nutrition of the fish is at this time purely speculative. It would seem that its determination may lie in the field of biochemistry, either by the detection of some substance developed under the con-

[3] *Jour. Am. Med. Assn.*, 1910, liv, 227.

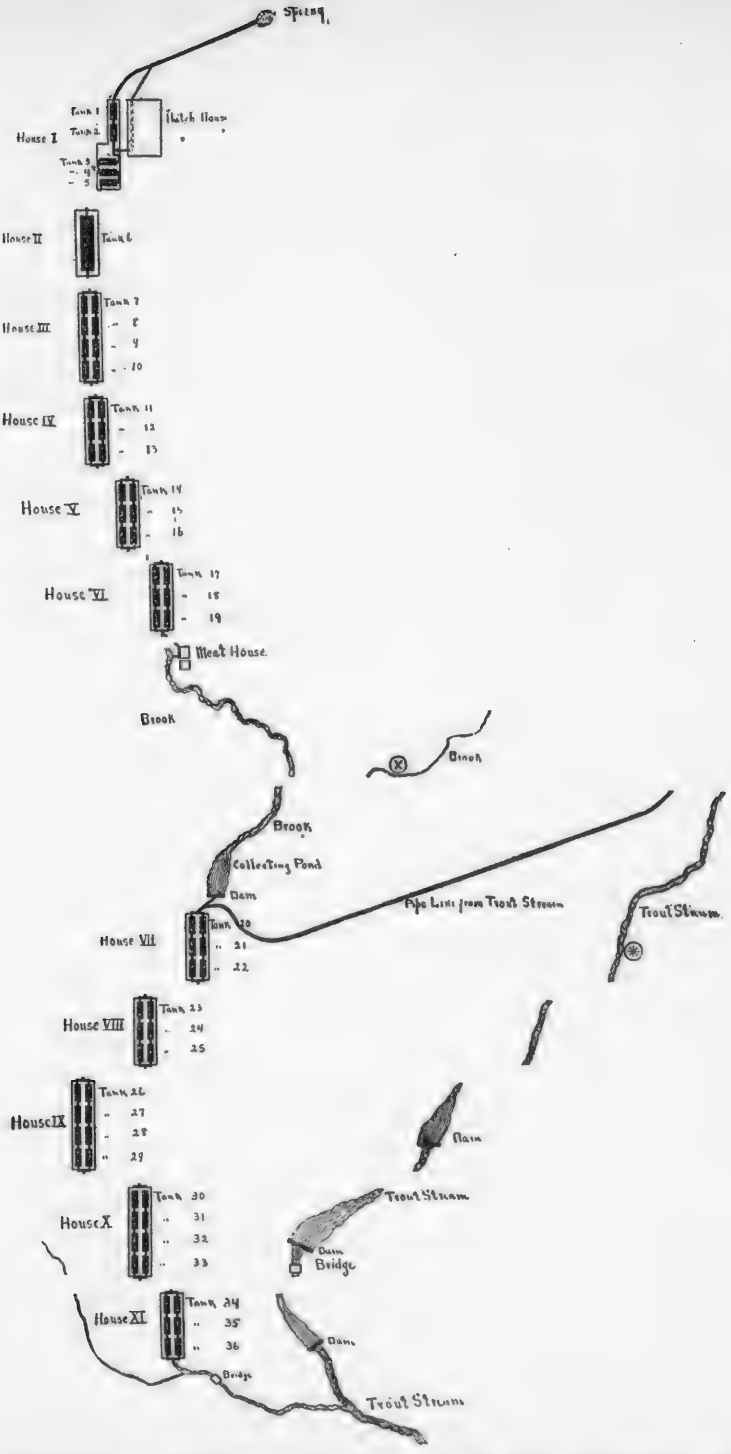
ditions of food, environment, and overcrowding, which the thyroid growth attempts to counteract, or by showing that the water contains salts necessary for the normal nutrition of the fish in sufficient amounts under natural conditions, but insufficient under these highly artificial conditions.

The influence which food may have is also at this time speculative, as little is known of the food requirements of these fish. Liver or heart muscle is a highly abnormal diet and is probably deficient in many of the elements necessary for normal nutrition. When fed in excessive amounts, it is conceivable that it may be injurious from the excess of certain, as well as from the deficiency of other, elements. Iodin has a preventative and curative effect on these early hyperplasias. The explanation of the action of iodin would probably explain the cause of thyroid hyperplasia. It may be stated that there is a deficiency of iodin in all actively hyperplastic thyroids, but it is not known where the iodin originates or what are the underlying factors which bring about this condition.

OBSERVATIONS ON THE HISTOLOGICAL STATE OF THE THYROID GLANDS IN A SERIES OF FISH REPRESENTING SPECIMENS FROM ALL TANKS (PONDS).

Similar observations were made in October, 1909, when the ages of the fish were thirty-two, twenty, and eight months respectively. In June, 1910, the ages were twenty-nine, seventeen, and five months, the three year old fish having been transferred to the trout stream in April, 1910, and the two and the one year old fish moved down to take their places and to make room for the 1910 fry. Apart from the different ages of the fish and their changed location, there were but two general conditions markedly different from those obtaining in 1909: (1) season (summer vs. autumn) and (2) a decreased water supply owing to the pipe line not being in operation for the past six months. It was thought that this second examination might offer additional data concerning any seasonal influence or the effect of a lessened water supply, and also serve as a control for the observations made in October.

The histological examination of the thyroids of one hundred and five trout showed that they all had well marked hyperplasia. The



TEXT-FIGURE 1. Semidiagrammatic drawing of the plan, arrangements, and location of the hatchery, water supplies, houses, and retaining tanks.

thyroids of the upper division (houses I to VI, see text-figure 1) were less hyperplastic than at the first examination in October, while the thyroids of the lower division (houses VII to XI) were more markedly hyperplastic than in October.[4]

The food both as to quantity and quality was the same as last year. The crowding of the fish was probably not so great. The water supply to the first six houses of the series was increased probably to a third more than that available in October. This was due to a decreased flow from the spring during the months of July, August, September, and October. We believe that the increased water supply during the winter and spring months largely explains the better condition of the fish thyroids in houses I to VI in June than that which existed in October, 1909.

In the lower division (houses VII to XI), it will be recalled that in October, with the pipe line in operation, the thyroids were undergoing spontaneous involution, while in June, with the pipe line out of operation for the past six months, the thyroids in general were more hyperplastic than those of the upper division, with the exception of house VII, which is the first house the water enters after its passage along the original brook bed from the upper division, and to which a small spring is added. In this house (VII) the thyroids are noticeably less active as judged by the standards above mentioned, and it is our opinion that the passage of the water along the brook and its slight increase in volume favorably alter in some unknown manner the effect on the fish. We also look upon the absence of additional water supply from the pipe line for the past six months as an important factor in bringing about the much more active thyroid hyperplasia present throughout the lower division than existed at the examination in October. There was no evidence of any noteworthy seasonal effect. It is generally stated that in mammals the thyroid tends to be less active (hyperplastic) during the winter months.

Summing up these observations, one may say that they offer

[4] The degree of hyperplasia is judged by the amount of stainable colloid and the size of the epithelial cells, and not by the total amount of thyroid tissue or its extension into the surrounding structures, which is present in all of these hatchery fish.

further evidence that increasing or decreasing the water supply modifies the condition of the thyroid. We are still ignorant of how an increased water supply lessens the thyroid hyperplasia. It may be that the salts in solution are necessary for normal nutrition and that their reduction disturbs the physiological balance, or that the susceptibility to some infectious agent is decreased by dilution, or by improving the fish's general nutrition.

OBSERVATIONS ON THE HISTOLOGICAL CONDITION OF THE THYROIDS OF THE TROUT OBTAINED FROM THE RUNWAYS ABOVE, BETWEEN, AND BELOW THE SEVERAL TANKS (PONDS).

Of this series of twenty-five trout, varying in age from seventeen months to four years, five were taken from the brook above all houses, sixteen from the runways within and between the eleven houses, and four from the tailrace below all houses. Of the five taken from the brook above all houses, one was twenty-nine months old and four were seventeen months old. They were normally colored, strong, vigorous fish and had spent their entire lives in this space of about fifty yards. All had normal thyroids both as regards the individual units, the follicles, and the total amount of thyroid tissue. They had not been fed or cared for in any way.

Of the sixteen fish taken from the wooden runways within and between the several houses, seven were considered as being seventeen months old, three as twenty-nine months old, and six as being over three years old. None of these fish had normal thyroids. In all there was more or less extensive spreading of the thyroid tissue beyond the normal area. In eleven of the fish the thyroids were in the colloid or resting stage, i. e., the individual follicles contained normally staining colloid and the epithelium was normal in type, although the total number of follicles was greatly increased above the normal. The remaining five fish had mild degrees of active hyperplasia. These sixteen fish had escaped from the ponds, but there was no way of ascertaining how long they had lived in the runways except by their size and the development of the color pigment in the skin, which indicated a stay of many months, and, in some instances, of years.

The four trout taken from the tailrace below the last house were

fish that had been transferred to the trout stream not later than April 15, 1910, and had, therefore, spent at least two months, and perhaps longer, outside the ponds. All were large three year old fish with darkly pigmented bodies. Two of these had pure colloid goitres, and in the remaining two involution was not complete.

Summing up the observations in this series, it is seen that the trout which lived in the raceway above all houses and which had never been confined in the ponds or fed or cared for in any way, developed normally and maintained normal thyroids throughout their lives, while fish not a yard away, but confined in the ponds, artificially fed, and overcrowded, quickly developed marked thyroid hyperplasia.

Next, it is seen that fish taken from the small plank troughs which conduct the water from one pond to another may have colloid or quiescent thyroids, that is they may recover from the hyperplasia present at the time of their escape from the ponds, even though fish in ponds above and below them are at the same time developing thyroid hyperplasia. Such observations do not accord with our present conceptions of a water-borne infectious agent as the etiological factor. On the other hand, certain of these fish had active hyperplasia, showing that the same factors which operate within the ponds are also operative in the raceways, but to a lesser degree.

Lastly, fish taken from the tailrace show the same tendency toward recovery, although living in the theoretically most polluted water. No general conclusion can be drawn from these observations beyond the negative evidence against a water-borne infection as the cause of goitre, and in favor of the interaction of several factors of a chemical nature influencing the nutrition of the fish.

OBSERVATIONS ON THE HISTOLOGICAL CONDITION OF THE THYROIDS OF THE TROUT TAKEN FROM THE TROUT STREAM.

There were eighteen trout in this series. All of these fish had spent at least two months in the stream. Two of them had the gross appearance of native trout, and as their thyroids were histologically normal, it may be concluded that they had never been confined in the ponds. The remaining sixteen fish had enlarged thyroids.

Three had small external goitres. In most of the cases the thyroid had returned to the pure colloid state, but in certain specimens the involution was not complete.

To sum up this series, it appears that fish hatched and reared in the stream do not develop thyroid hyperplasia, and also that fish whose thyroids undergo marked hyperplasia during their stay in the ponds (without exception, so far as our observations on fifty-two cases are concerned) proceed to spontaneous recovery when transferred to the trout stream. We have also examined pickerel, suckers, and cat fish from this stream and have never observed abnormal thyroids in them.

PART II.

EXPERIMENTAL OBSERVATIONS.

These experiments were undertaken to supplement the experiments made in October, 1909, when the effects of iodin on mild degrees of hyperplasia[5] were studied, and to extend these observations to include the severest grades; namely, fish with large, ulcerated, ventral goitres. The experiments can be divided into three series. In the first, was studied the effect of iodin on forty-one months old fish, with large, ulcerated, ventral goitres and with general emaciation; in the second, the effect of iodin on twenty-nine months old fish with small, visible goitres, but otherwise of a clean, healthy appearance; and in the third, the effect of the non-administration of iodin on twenty-nine months old trout with small ventral goitres.

[5] The first constant and readily detectable clinical sign of thyroid hyperplasia is the reddish discoloration of the floor of the pharynx. This reddening depends on the extension of the highly vascular thyroid tissue up to the mucosa of the pharynx and the transmission of the color of this tissue through the translucent mucosa. The extent of the area of reddish discoloration is a good clinical index of the extent of the thyroid overgrowth, the color of the normal pharyngeal floor being white or grayish white. As the hyperplasia changes to the colloid or quiescent state during recovery, the vascularity decreases, and with it the reddish color fades to a yellow or yellowish brown which persists throughout the life of the fish. The coloration depends upon the thyroid follicles with their colloid contents.

Experiment I. Effect of Iodin on Large Ulcerated Goitres.[6]—Twelve forty-one months old, emaciated brook trout, instead of being destroyed, as is usually done, were saved for this purpose when the three year old fish were transferred to the trout stream in April. On June 20, they were placed in a cypress trough 13 feet long, 20 inches wide, and 8 inches deep. The water used was taken directly from a spring marked X on the diagram (text-figure 1). Its temperature was 48–50 degrees F. The flow through the trough was adjusted so that it was sufficient to fill the trough in one hour. All the fish had large ventral ulcerated and bleeding goitres. Three had gill fungus and died during the experiment. Of the remaining nine, five were sacrificed on the fortieth day, and four on the sixty-second day. The food consisted of about three ounces of finely chopped hog's heart or liver given each morning, and on three occasions during the first forty days live minnows were given. One cubic centimeter of the U. S. Pharmacopeia tincture of iodin (potassium iodid 5 grams, iodin 7 grams, alcohol (95 per cent.) to 100 c.c.) was added to the trough contents each morning before feeding. The trough was located in a well shaded gully and wire cloth was fastened about it to prevent the fish from jumping out. Once each week the debris (leaves, uneaten food, and feces) was removed from the trough by flushing. The examination of the nine fish at the time of removal revealed remarkable changes in their appearance. They had all gained greatly in weight. They had lost much of their dark pigment, and the color pigment had developed in the fins and along the sides of the fish. They were lighter in color than the native trout, having the silvery sheen approaching that of the true salmon. They were all robust clean fish. Of the five removed on the fortieth day, three still had visibly flattened and ulcerated goitres. Of the four removed on the sixty-second day, only one had distinct ulceration, the ulceration on the others having practically healed.

The histological condition of the thyroids of these nine trout is given in table I. Of the five removed on the fortieth day, all showed evidence of infection. In two, the infection had nearly resolved. The stroma was increased in all, probably abnormally so on account of the infection. In the more protected follicles to which the infection had not extended, or in which it had cleared up, there was

[6] Ulceration and infection are very common complications of the large goitres. The most important causes predisposing to them are: (1) Injury. The goitre being the most prominent point on the ventral surface of the fish is exposed to frequent trauma, especially in the confined quarters of the ponds. (2) As the goitre enlarges, the skin over it is thinned by pressure atrophy, and perhaps a break in continuity may occur from this cause alone. When once the skin is broken, infection is practically unavoidable on account of the rich bacterial flora of the ponds and because of the increased susceptibility of these fish which the growing goitre implies. After infection occurs, the necrotic tissue is worn away by friction, so that as we see these goitres they often have flat ventral surfaces practically in a plane with the ventral surfaces of the bodies of the fish. Therefore it follows that by placing such fish in shallow troughs for experimental purposes the chances of healing are reduced, because the exposed surface of the goitre is frequently rubbed against the bottom. The occurrence of infection in fish goitre is perhaps the most important and most frequent complication, and should be considered in any interpretation of histological data.

TABLE I.

No. of fish.	Duration of iodin treatment.	Histological condition of thyroid gland.				Diagnosis and general remarks.
		Distribution, infiltration.	Stroma.	Stainable colloid.	Epithelium.	
1	40 days	Large ulcerated goitre; all tissues beneath pharyngeal mucosa infiltrated; disappearance of bone and muscle.	Markedly thickened throughout; densely infiltrated with leucocytes; areas of abscess formation.	In the more protected areas just beneath pharyngeal mucosa and along gill arch arteries are areas with well stained colloid and but few leucocytes.	Flat cubical in the involuted areas; little distinguishable thyroid epithelium in the infected area; one papillo-adenoma unaffected by iodin.	This is an example of a severely ulcerated goitre with marked general inflammatory reaction giving the appearance in places of sarcoma. Most of the thyroid follicles are filled with leucocytes—involution, general infection, and papillo-adenoma.
2	40 days	Large ulcerated goitre; much thyroid tissue throughout which has extended into all subpharyngeal tissues; absorption of bone and cartilage; compression of aorta.	Everywhere thickened and densely infiltrated with leucocytes throughout most of goitre.	Well preserved follicles contain colloid. Entire lower portion is an infiltrated mass of leucocytes and granulation tissue.	Cubical in the preserved portions, while in the infected area the epithelium is not clearly distinguishable.	Old infected goitre with destruction of most of the true thyroid tissue by the inflammatory process.
3	40 days	Large, flattened, nearly healed goitre; all subpharyngeal tissues infiltrated; absorption of bone, muscle, and cartilage; compression of aorta; thyroid growth at tip of lower jaw.	Increased throughout, especially in the exposed parts.	Colloid normal throughout goitre; leucocytes in the colloid of the exposed and inflammatory area.	Cuboidal in general but columnar on the crests of many of the infoldings and particularly in the papillo-adenomatous area.	Rapid involution of goitre with healing of the infection; here is an adenomatous area that is beginning to change; the submaxillary thyroid tissue has changed to the pure colloid state.
4	40 days	Large ulcerated goitre; all subpharyngeal tissues infiltrated.	Everywhere increased; much cellular exudate throughout ventral portion of goitre.	All follicles of protected areas contain colloid. Those in the infected area are ill-defined and filled with leucocytes.	In general cuboidal in the uncomplicated areas but columnar in the infected area.	Example of involution to the colloid state occurring in a mildly infected goitre; infection resolving.

TABLE I. (*Continued.*)

No. of fish.	Duration of iodin treatment.	Histological condition of thyroid gland				Diagnosis and general remarks.
		Distribution, infiltration.	Stroma.	Stainable colloid.	Epithelium.	
5	40 days	Small non-ulcerated or healed goitre; all tissues beneath pharyngeal mucosa invaded; absorption of bone.	Markedly increased throughout—probably largely scar tissue.	Follicles small, colloid-containing; few leucocytes still present in certain follicles.	Cuboidal throughout.	Example of nearly complete involution with healing of the ulcerated and infected goitre.
6	62 days	All subpharyngeal tissues involved; little normal thyroid present; absorption of bone and compression of aorta.	Granulation tissue overgrowth; inflammatory exudate throughout stroma.	Colloid follicles scattered through dorsal part of goitre	Cuboidal in the colloid-containing follicles. Much of the thyroid tissue replaced by cellular exudate and granulation tissue.	Example of slight involution in 62 days. This is probably due to the marked infection and illustrates the disturbing influence of infection on involution.
7	62 days	Small healed goitre; all subpharyngeal tissues invaded; absorption of muscle and bone.	Everywhere increased, especially in ventral portion.	Follicles small; colloid-containing throughout.	Cuboidal regular, and uniform.	Example of complete healing of infected area and of complete involution to the colloid state.
8	62 days	Healed goitre; all subpharyngeal tissues invaded including bone and cartilage.	Very dense—probably result of old infection.	Follicles very small; contain normal colloid; one area with marked infoldings of lining epithelium and containing very little colloid-like material.	Cuboidal throughout the colloid-containing portion; the adenomatous area still has high columnar epithelium.	Example of involution and healing of an infected goitre. The goitre also contains an adenoma with papillomatous ingrowths which has been slightly modified by the iodin in the 62 days.
9	62 days	Healed goitre; all tissues beneath pharyngeal mucosa invaded and also small nodules projecting into pharynx; absorption of bone and muscle.	Slightly increased.	Follicles everywhere contain colloid; some exudate in places in stroma.	Cuboidal, regular, uniform.	Example of healing and involution of pure type of hyperplasia, where the only complication was infection.

the normal accumulation of colloid with the return of the follicular epithelium to the flat cubical type. Throughout the infected areas the follicles contained large numbers of leucocytes and shed epithelial cells, and the stroma was cellular, giving the appearance of much new connective tissue formation (granuloma) together with an infiltration of leucocytes. This appearance at first gives one the impression of a connective tissue tumor rather than of thyroid granulation tissue. In two there were somewhat circumscribed areas with very striking papillomatous outgrowths and infoldings of the lining epithelium. In one of these tumor nodules, iodin had had no detectable effect toward involution, while in the second and less extensive papillomatous growth, iodin had stopped its growth and brought about the early changes of involution, i. e., beginning colloid formation and shrinkage in the size of the epithelial cells. These more or less circumscribed areas of rapidly growing thyroid tissue, with a tendency toward arborescence, are frequently seen in fish goitres, and, as they have many of the attributes of true thyroid tumors (localized, rapid growth, atypical follicle development, and slight or no reaction with iodin), we look upon them as true tumors; and, until something suggestive of malignancy can be recognized, as benign tumors comparable to the ordinary tumors in human goitres. Of the four trout removed on the sixty-second day, one had a papillomatous tumor with complete involution of the surrounding original hyperplasia while the tumor was only slightly modified, again suggesting that in such cases the thyroid cells have lost certain of the attributes of ordinary hyperplastic thyroid cells. All of these sixty-two day fish thyroids showed practically complete involution, with nearly complete healing of the infections.

Summing up this experiment, the more striking features are: (1) the remarkable improvement that may take place in these fish in a short time; (2) the extent and frequency of infectious processes in fish goitres, and the influence which they exert in retarding involution from iodin; (3) the presence of adeno-papillomatous areas, in three of the nine cases, that resemble in many respects true thyroid tumors. These nine cases represent the very advanced stage of fish goitre. Such fish are always destroyed by the caretaker when the transfers are made, because it is popularly believed that they can not recover. This is erroneous. They can and do recover in most instances when sufficiently favorable hygienic conditions are instituted. The goitre does not ordinarily disappear as the fish recovers, for the same reason that it does not disappear with recovery in mammals. It does, however, change to the colloid or quiescent stage, just as in mammals, and this is the nearest approach to the normal state possible for them. There is, therefore, no biological evidence that these severe grades of goitre are cancerous.

TABLE II.

No. of fish.	Duration of iodin. treatment.	Histological condition of thyroid gland.				Diagnosis and general remarks.
		Distribution, infiltration.	Stroma.	Stainable colloid	Epithelium.	
1	40 days	Entire space beneath pharyngeal mucosa infiltrated with thyroid tissue; absorption of bone, cartilage, and muscle; ventral surface of goitre worn away by friction and ulceration.	Generalized increase; also extensive cellular exudate and granulation tissue formation.	Many follicles with normal colloid throughout goitre; extensive areas where follicles contain shed epithelium and leucocytes with little true colloid.	Flat cubical in all follicles with normal colloid; columnar in the inflammatory areas; one area has arborescent outgrowths of lining epithelium (tumor).	Involution to colloid state well advanced. The ulceration and inflammatory reaction are complicating and retarding factors. One large circumscribed papillomatous area (adenoma).
2	40 days	Entire space beneath pharyngeal mucosa infiltrated with thyroid tissue including bone and cartilage	Slightly increased, especially about bone and skin.	Follicles generally enlarged and filled with normally staining colloid.	Uniform, flat, cuboidal.	Complete involution to colloid state. No complicating factor.
3	40 days	Entire space beneath pharyngeal mucosa infiltrated with ulcerated thyroid tissue.	Marked increase in stroma and extension of inflammatory reaction throughout goitre.	Follicles in less inflamed portion are filled with colloid; in the main mass of the goitre the follicles contain leucocytes and granular albuminous debris.	Throughout the more infected areas the follicles have columnar epithelium, while the remaining follicles have cuboidal epithelium.	Involution well advanced in the areas free from inflammatory reaction.
4	40 days	Entire space beneath pharyngeal mucosa invaded with thyroid tissue, including bone and muscle.	Slight general increase.	Follicles enlarged and show infoldings of the lining epithelium; colloid normal.	Cuboidal throughout.	Complete involution of a small uncomplicated goitre.
5	62 days	Entire space beneath pharyngeal mucosa invaded and infiltrated with thyroid.	Well marked increase.	Follicles small, rounded and filled with colloid.	Cuboidal.	Complete involution. No complication.

TABLE II. (*Continued.*)

No. of fish.	Duration of iodin treatment.	Histological condition of thyroid gland.				Diagnosis and general remarks.
		Distribution, infiltration.	Stroma.	Stainable colloid.	Epithelium.	
6	62 days	Small nodular growths of thyroid tissue elevating the pharyngeal mucosa. All tissues beneath mucosa invaded, extensions along gill arch arteries.	Slight increase.	Follicles enlarged and filled with colloid.	Cuboidal.	Complete involution to colloid state. No complication. Accessory thyroid growth at tip of lower lip showing complete involution.
7	62 days	Entire subpharyngeal space filled with thyroid tissue; erosion of bone and cartilage.	Stroma increased about more resistant structures.	Follicles large in the body of the goitre but small and separated by stroma bands at periphery; colloid normal.	Cuboidal.	Complete involution. No complication. Some evidence of a previous mild infection.

Experiment II.[7] *Effect of Iodin on Thyroid Hyperplasia of Twenty-Nine Months Old Fish with Small Ventral Goitres but Otherwise of Healthy Appearance.*—This experiment was carried out in exactly the same way as experiment I. The trough was of the same size and situated alongside of that used in experiment I. The water supply, both as regards quality and quantity, the food, the amount of iodin, and the general care were similar. Seven trout were placed in the trough on June 20, 1910. Four were removed on July 30, and three on September 1, 1910. They had all gained greatly in weight. Much of the dark skin pigment had disappeared. The orange colored pigment in the fins and the yellow-red spots along the sides of the body had developed. The pharyngeal mucosa had lost much of the original reddish discoloration, and the ventral goitres, which at the beginning of the experiment varied in size from 3 to 6 mm. in diameter, became much smaller and of a yellowish translucent appearance, showing that the fish goitre in common with the mammalian goitre usually becomes smaller as the involution to the colloid state takes place. Two trout had ulcerated and infected goitres, probably the result of rubbing against the trough walls.

The histological condition is tabulated in table II, and may be summarized as follows:

In all specimens, the thyroid tissue had infiltrated all the structures beneath the pharyngeal mucosa. There were extensions into the gill arches along the arteries. The adjacent muscle in some cases was totally replaced by thyroid tissue. There was much absorption of the adjacent bony and cartilaginous parts. While the thyroid tissue completely surrounded the aorta, there was no case where it had extended beyond the adventitial coat. There was often compression of the aorta and its branches by the adjacent thyroid tissue. In the two infected specimens there was a very marked increase in the stroma, which was probably largely of inflammatory origin. The stroma is normally increased in active hyperplasia, being more pronounced about the more resistant structures, such as the bone, skin, and arteries. With the exception of the ulcerated goitres, the follicles all contained normally staining colloid. The epithelium had returned to the normal cubical form, and the blood supply was reduced. In one case there was a small accessory goitre at the tip of the lower lip, which upon histological examination showed complete involution to the colloid state, as was the case of the thyroid proper. This is the best or biological evidence that these submaxillary growths are not metastases, but embryologically misplaced thyroid anlagen.[8]

[7] Control experiments with trout can not be carried out as with mammals for the reason that the hemorrhage and infection consequent upon removing portions of the goitre can not be controlled. We therefore took as controls five fish of the same age and from the same pond, showing small ventral goitres. The histological examination showed well marked active hyperplasia in all these specimens.

[8] We have never observed true thyroid metastases. By some observers the submaxillary growths have been looked upon as metastases. They were present in 2 to 3 per cent. of all fish with visible ventral goitres examined by us. We have observed one instance of an abdominal goitre in a twenty-nine months old

Taking the experiment as a whole, we find that these mild degrees of visible goitres are affected by iodin in the same way as the still earlier stages, before visible goitres appear, and hence are not true tumors, but merely the more advanced stage of the common goitrous process.

Experiment III. Effect on the Thyroid Glands of Twenty-Nine Months Old Fish (Trout) of a Residence of Thirty-Eight Days in the Trout Stream.—A pond was made in the trout stream (location marked * in text-figure 1) by fencing off with wire cloth a portion of a pool forty feet long where an overhanging bank afforded cover and shade for the fish. This also allowed free entrance and exit for the passing water, and closely approximated the natural environment of the native trout. Fifteen twenty-nine months old fish with very small ventral goitres, but otherwise of healthful appearance, were transferred on June 22 from the ponds to the enclosure above described. No iodin was used and the only food given directly was some live minnows on three occasions. On July 30 we were able to recover eight of these trout. Their general color was perhaps somewhat brighter than at the beginning of the experiment, and there had occurred a slight development of the orange and yellow skin spots. All were active, clean, vigorous, well nourished fish. The pharyngeal mucosa was still somewhat reddish in color and the ventral tumors were still visible.

Histological examination of these eight fish showed the usual generalized invasion of all the subpharyngeal tissues, with extensions to the gill arches. In general the follicles were large, but about the more resistant structures where the stroma is normally dense, they were small. All the follicles contained stainable colloid, but in none had it returned to its normal staining. The epithelium lining the follicles was still columnar or high cubical. When these fish were compared with the controls, however, it was found that a great change had taken place in the thirty-eight days. That is, the thyroids were changing to the colloid stage, but the process was still far from complete. This slow change was to be expected since we had uniformly found the same changes occurring in the thyroids of the trout taken at random from the trout stream after their residence in the stream for varying lengths of time.

When this experiment is considered in connection with experiments I and II, it is seen that spontaneous involution (recovery) of the thyroid is much slower than the induced involution from iodin. It will be recalled in this connection that similar differences between the spontaneous involution and the induced involution

fish. The thyroid mass was round and encapsulated and measured 1 by 1.2 cm. It was attached to the cardiac end of the stomach by a connective tissue pedicle. This fish also had a large ventral goitre histologically identical with the abdominal growth. Careful examination of the visceral organs did not show any thyroid tissue. We therefore look upon this specimen as an enlarged aberrant thyroid like the submaxillary growths.

from iodin occur in mammals. However, the end stage of both processes, namely, the colloid gland, agrees anatomically and physiologically in both groups.

SUMMARY.

In a series of observations upon the thyroids of brook trout, extending from the time of hatching to fish of four or more years old, we have been able to follow step by step the development of thyroid hyperplasia. In these hatchery trout, thyroid overgrowth may be recognized anatomically at the end of the second month of extra-oval life. Under the conditions in which these fish live, the thyroid overgrowth progressively increases, at first filling the aortic space and then invading the surrounding bone and muscle. In our series, this extension into the adjacent tissues was not recognizable until the fourth month of extra-oval life. The time when invasion of the surrounding tissues is first noticed is, of course, subject to great variation in different years and different hatcheries, depending on the general hygienic conditions, food, water supply, etc. If the growth continues, as it usually does, throughout the rapid growing period of the fish, it may first be detected clinically by a reddening of the pharyngeal floor as the thyroid tissue spreads to the submucosa. Later, definite external goitres appear ventrally. These may be present as early as the sixth month of life. The thyroid being, as in mammals, most active during the growing period, the greatest number of visible goitres appear during the second and third years of life. In older fish the thyroid again becomes less active and there is a tendency toward spontaneous recovery. There is no noteworthy change in the anatomical appearance of the thyroid growth, whether seen in very young or in older fish, other than that clearly dependent on the age of the fish, mechanical factors, as size of the goitre, and complicating factors, as infection, hemorrhage, degeneration, etc. There exists, therefore, no anatomical basis for the diagnosis of cancer in the older fish that is not also present in the youngest fish with thyroid hyperplasia. All the appearances of invasion, atypical cell growth, etc. are the results of the progressive growth and consequent extension of a non-encapsulated epithelial tissue along the paths of least resistance.

In many of the larger goitres there are more or less distinct areas of thyroid hyperplasia, histologically different from the surrounding thyroid tissue, which we look upon as benign tumors comparable to the benign tumors seen in human goitres. We class them as tumors because they do not react with iodin as does the ordinary hyperplasia. To call these tumors cancer is going beyond our present knowledge and is unjustified, even though analogy with mammalian tumors suggests that certain ones might proceed to true carcinomata. The most common type of the benign tumors is the arborescent-papillomatous form and its many modifications.

Infection is a frequent complication in the larger goitres, for the reasons given. It is necessary to recognize this factor in interpreting the histological conditions met with, as otherwise, using mammalian standards, some of the appearances observed could easily be mistaken for sarcoma.

The effect of iodin on the thyroid hyperplasia has been studied in the early, middle, and late stages. All stages react with iodin, the mild degrees seemingly more rapidly than the severer degrees. Thus the early stages undergo involution in from two to three weeks, while the late stages may require one to two months. The process, therefore, is slower than the iodin reaction time in mammalian hyperplasia. True tumors do not react with iodin as does ordinary hyperplasia. Infection or other complication modifies the reaction. The reaction with iodin is a specific test for functional hyperplasia of the thyroid. As we have not been able to find any stage in the process that does not react with iodin, we must conclude that there is none that may be looked upon biologically as cancer. Just as iodin invariably stops the hyperplasia and causes the thyroid to return to the colloid or resting state (from which it may undergo hyperplasia a second, third, or more times, exactly as in mammals), so also spontaneous involution occurs when the fish are transferred to a natural environment. This spontaneous involution has been followed in experiments and has been seen in examples taken at random from the streams. We have never seen an exception to this rule, save in the case of true tumors. As pointed out above, recovery does not imply a disappearance of the goitre;

it implies a cessation of growth and return of the active hyperplasia to the colloid or resting stage.

Thyroid hyperplasia is a compensatory reaction, the exact cause of which is still to be sought. There is no evidence that in fish or in man it is either infectious or contagious. All the biological data at present available favor the view that fish goitre in common with mammalian goitre is the symptomatic manifestation of a metabolic and nutritional disturbance. There are three major conditions which, in some way still obscure, influence the thyroid growth; namely, a *limited water supply, overcrowding,* and *overfeeding* with a highly artificial and incomplete food. The water of the hatchery observed is not intrinsically goitre-producing, as the fish will not develop goitre unless at least the factor of overfeeding with an incomplete food operates at the same time. On the other hand, they recover if the overfeeding and overcrowding are corrected, although they remain in the same water. Therefore it seems probable that the *food* is the major factor acting to bring about a fault of nutrition favorable for goitre development. It is impossible at this time to suggest what elements in the food may be at fault, whether, for example, it is deficiency or excess or disproportion in the relative food values.

CONCLUSIONS.

1. There is no stage of thyroid hyperplasia in brook trout that may be classified biologically as carcinoma.

2. The incidence of true carcinoma in fish goitre is not greater than in mammalian goitre.

3. There is no evidence that goitre is either infectious or contagious.

4. Goitre is endemic in all hatcheries where the *salmonidae* are artificially reared. Its severity is quantitatively related to the general hygienic conditions prevailing, and to the food, water supply, and degree of crowding.

5. The immediate cause of goitre is unknown, but it depends in all probability on a disproportion in, or a lack of, certain of the elements necessary for proper nutrition.

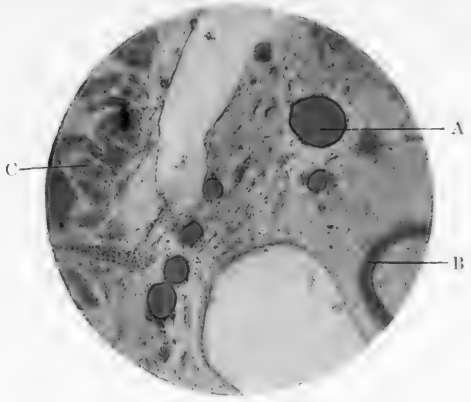


FIG. 1.

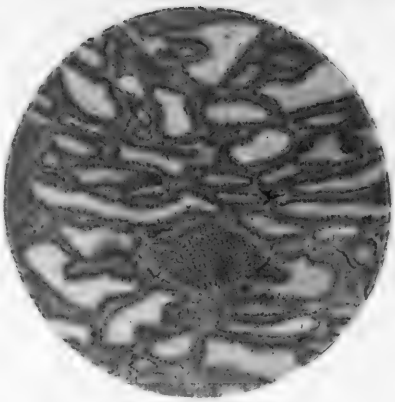


FIG. 2.

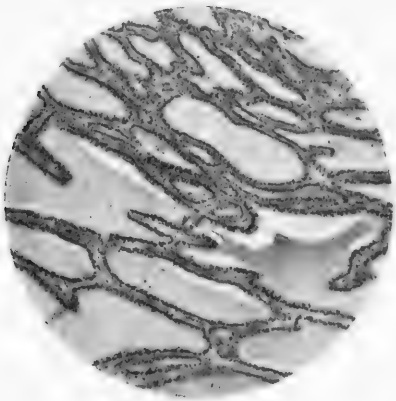


FIG. 3.

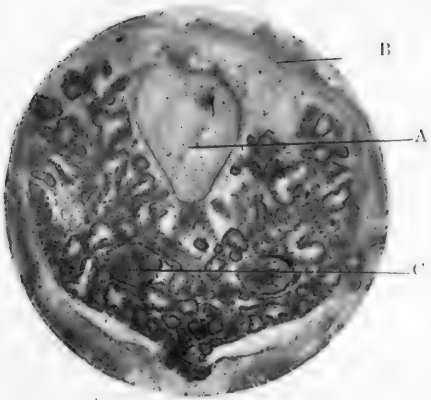


FIG. 4.

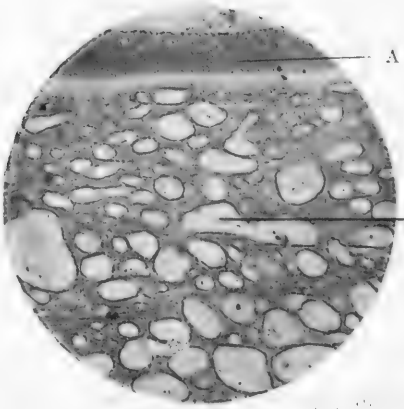


FIG. 5.

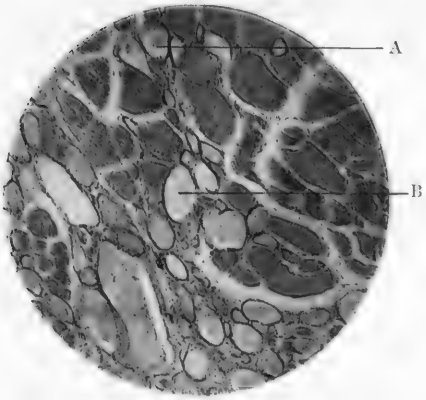


FIG. 6.

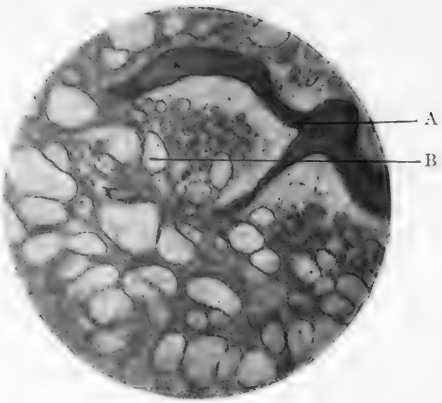


FIG. 7.

FIG. 8.

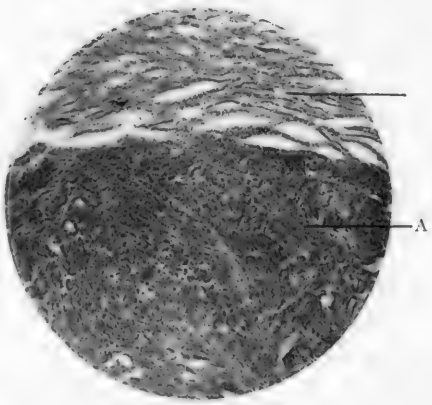


FIG. 9.

This study was made possible through facilities granted by and through the coöperation of the Honorable W. E. Meehan, Commissioner of Fisheries for the State of Pennsylvania.

We wish to express our obligations and thanks to Mr. Meehan, to the officers of the Blooming Grove Hunting and Fishing Club, and to Mr. Florence Rolonson, Superintendent at the hatchery, for their aid and interest.

EXPLANATION OF PLATES.

PLATE LX.

FIG. 1. Normal thyroid from a twenty-nine months old brook trout, showing the distribution of the follicles. A = thyroid follicles, B = aorta, C = striped muscle bundle.

FIG. 2. Developing goitre (active thyroid hyperplasia). Note the enlargment and distortion of the follicles, the regular, uniform columnar epithelium, and the great reduction in the colloid content of the follicles.

FIG. 3. The beginning of the process of recovery (involution), or the return of the active hyperplasia (as shown in Fig. 2) to the colloid or resting stage. The elongation of the follicles in this case is due to tension. Note the appearance of stainable colloid in the follicles, the contraction of the stroma and early regressive changes in the follicular epithelium.

FIG. 4. Thyroid area of a five months old brook trout. Note that the entire thyroid area is filled with actively growing thyroid tissue with the usual follicular infoldings and columnar epithelium characteristic of active thyroid hyperplasia. A = bone and cartilage, B = pharyngeal mucosa, C = the two terminal branches of the ventral aorta.

FIG. 5. Colloid goitre as the result of iodin treatment of active thyroid hyperplasia. Note the flat cubical epithelium, the rounded colloid-containing follicles, and the much increased stroma. A = pharyngeal mucosa, B = thyroid follicles.

PLATE LXI.

FIG. 6. Colloid goitre—spontaneous involution due to residence in the trout stream. Note the similarity of the individual follicles as regards epithelium and colloid to those of Fig. 5. Note also the extension of the follicles between the muscle fibres (so-called invasion). A and B = thyroid follicles in the striped muscle bundle.

FIG. 7. Colloid goitre—effect of iodin on active hyperplasia. Note the relation of follicles to bone. A = bone, B = thyroid follicles with normal colloid and epithelium.

FIG. 8. Portion of thyroid area of six months old fry, showing complete filling of thyroid area with actively hyperplastic thyroid tissue. A = relation to ventral aorta, B = relation to bone and cartilage, C = relation to striped muscle, D = relation to vein, E = gill arch.

FIG. 9. Portion of large ventral goitre, showing (A) growing border of a thyroid tumor, and (B) adjacent thyroid hyperplasia in which the tumor developed.